Ancient Science of Life, Vol No. X No. 1 July 1990, Pages 56– 58

AROGYA VARDHII BATI AND ANAND BHAIRAVA RAS, TWO AMOEBICIDAL AYURVEDIC DRUGS

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Received: 15 September, 1989 **Accepted: 20 November, 1989**

ABSTRACT: *Two ayurvedic preparations Arogya Vardhini Bati (AVB) and Anand Bhairava Ras (ABR) were found to cure intestinal amoebiasis of rat and hepatic amoebiasis of golden hamster. Both AVB and ABR were found to be amoebicidal against trophozoites of Entamoeba histolytic at 500 µg/ml in vitro and cured caecal amoebiasis of rat at 200 mg/kg/dose/5 dyas, in vivo.*

INTRODUCTION

For the treatment of parasititc diseases of man, ayurvedic preparations have been used from ancient times. With the development of animal model for human parasitic diseases, most of the synthetic drugs are tested both *in vitro* and *in vivo* to find out the efficacy of the compounds. Ayurvedic drugs are directly prescribed to humans for the treatment of diseases, as these drugs are generally supposed to be non-toxic. Confirmation of the efficacy of ayurvedic drugs by animal experimentation has not been in most of the drugs.

The present communication, therefore, reports the confirmation study, through animal experimentations, of two potent ayurvedic preparations, Arogya Vardhini Bati and Anand Bhairava Ras used in the treatment of amoebiasis of man.

MATERIALS AND METHODS

Amoeba : Axenically grown trophozites of *Entamoeba histolytics* (200 – NIH) and miexed bacterial culture of pathogenic *E. hostolytica* (strain H-39).

Animals : Albino rat, park strain and golden hamster, from stock of CDRI animal house, fed on food pellets, were used.

Drugs: 1) Arogya Vardhini Bati (AVB)[1]: Composition:- Mercury purified (^{0}part), Sulphur purified (1 part), Lauha Bhasma (1 part) Abhra bhasma; Tamra Bhasma; Triphala (6 parts): pure shilazeet (3 parts); *Commiphora mukul* (4 parts); Root of *Plumbago Zailanica; Picrorrhiza kurroa* (22 parts). Prepared in the leaf extract of *Melia azadirachta.*

ii) Anand Bhairava Ras (ABR)[2] Composition: - Cinnabar purified (1 part), *Aconitum ferox* (1 part); *Zingiber officinale, Piper nigrum* (1 part), *Piper longum* (1 part); Borax purified (1 part); Mace (1 part). Prepared in the extract of citrus lemon juice.

In vitro test: [3]

In the case of drug screening against axenically grown *E. histolytica,* trophozoites of 48 to 72 hr old cultures growing in

modified Diamond's medium4 were collected by centrifugation following the method of Das and Prasad[5]. 0.2 ml inoculum containing about 2,000 amoebae was put into cavity slide and filled with fresh medium (0.8 ml) containing the required concentration of the drug. The cavity was covered with a cover slip, and the edges of the cover slip were sealed with paraffin wax. The slides were put in a moist chamber at 37°C. Observations were taken after 6, 18, 24, and 72 hrs under inverted microscope to determine whether amoebae were dead or alive. In doubtful cases subcultures were made in fresh culture medium. Petri dishes serving as moist chambers were sealed from outside by adhesive tape to avoid contamination. In the case of the control group, no drug was added. Duplicate sets were run for each drug concentration.

In vitro test: [3]

1) Experimental caecal amoebiasis of rat

Albino rats (25 – 30 g) 3 weeks old and free from *E. muris* infection, were used. A virulent strain of *E. histolytica* (48 hr. old culture), growing on mixed bacterial flora plus rice starch in modified Boeck and Drbohlav (B & D) medium, was injected into the caecum of rat by technique described by Jones[6]. The inoculum (0.25 cc) containing about 80,000 amoebas, associated bacterial flora plus rice starch was given to each animal. The animals were divided into two groups. One group served as control and to the other was administered the drug orally (aqueous suspension of fine drug powder) through a catheter attached to 1 ml syringe. The curative effect of the compound was studied by giving it 48 hr after infection during which time the caecal wall is generally slightly ulcerated. The drug was given every 24 hrs for five successive days, after which the control and the treated animals were sacrificed. Caecal scoring was done on similar principle according to Neal's method[7] (wall normal = 0; slight thickening = 1; marked local thickening and contraction = 2; extensive thickening, contraction and slight ulceration = 3, caecum shapeless, extensive ulceration with abscess formation = 4); contents (normal = 0; thick reddish = 1, mucoid = 2, some solid matter present = 3; no solid matter, white or yellowish mucus = 4).

2) Experimental hepatic amoebiasis of hamster (Cricatus auratus[3])

Golden hamster, of either sex, weighing between 40 45 gm and approximately one month old were routinely used in this experiment. The animals were obtained from CDRI colony and kept on simple pellet diet.

For hepatic infection, animals were anaesthetized with ether and a 0.5 cm long incision in the abdominal skin was made a little below xiphisternum. Connective tissue underlying the skin was gently removed to expose the muscular layer of the abdomen with the help of a pair of a forceps. The muscular layer was raised from the viscera and 0.05 to 0.06 ml of the inoculum, containing 25,000 to 50,000 trophozoites were injected into the peritoneal cavity near the liver with a tuberculin syringe through the muscular layer. Boric acid powder was applied to the bleeding surface in an effort to control haemorrhage, and the body wall was sutured. This method ensures that the whole inoculum of amoebae was dropped in the peritoneal cavity in the liver region and not accidentally injected into the viscera.

Infected hamsters generally die within four to five days. The liver lesions were cultured and also examined microscopically. The

grading of liver lesion was done as described by Dutta (1970).

Grade 0 = No amoebic lesion, bacterial abscess may be present.

Grade 1 = Tiny superficial amoebic lesions covering upto 5% of liver surface.

Grade 2 = 5 to 15% of liver surface showing lesions or inflammation with superficial necrosis.

Grade 3 = 25% of liver showing lesions.

Grade 4 = Acute single or multiple lesions covering more than 25% of the liver surface with necrosis and pus, may be extending the whole thickness of one or more liver lobes.

When the infected liver tissue was cultured in modified B & D medium or in Robinson's medium[13] and examined microscopically, active trophozoites were present in all samples having grades one to four lesions[4].

RESULTS

The *in vitro* amoebicidal activity test revealed that both Arogya Vadhini Bati (AVB) and Anand Bhairava Ras (ABR) are amoebicidal at 500 µg|ml (Table 1). These drugs cured intestinal amoebiasis of rat at a dose of 200 mg | kg | rat | day | 5 days only 50% cure could be obtained in both the cases. 50 mg | kg does was found to be ineffective (Table 2). Control animals showed high rate of caecal ulceration (rat) and hepatic amoebiasis (golden hamster).

TABLE 1

Name of Drugs	*in vitro* amoebicidal activity of Arogya Vardhini Bat (AVB) and Anand Bhairava Ras (ABR) against axenic *E. histolytica*
AVB	Amoebicidal and point 1,2000 (500 µg | ml)
ABR	1,2000 (500 µg | ml)
Flagyl (Metronidazal)	4 µg | ml

TABLE II
***In vivo* anti amoebiasis activity against experimental intestinal amoebiasis of rat and hepatic amoebiasis of hamster of AVB and ABR.**

Name of drugs	**Dose mg | kg**	**No. of animals inoculated / infected**	**Av. Score of lesions**	
			Caecal	**Hepatic**
AVB	200	6 | 0	0.0	0.0
	100	6 | 3	3.0	2.0
	50	6 | 6	7.0	3.5

ABR	200	6 | 0	0.0	0.0
	100	6 | 3	3.5	2.0
	50	6 | 6	7.5	4.0
Control	-	6 | 6	8.0	4.0

DISCUSSION

Use of both Arogya Vardhini Bati[1] and Anand Bhairava Ras[2] for the treatment of human patients of diarrhoea and dysentery, have been reported in ayurvedic texts. Experiments using animal models to confirm the efficacy of these drugs against amoebiasis, caused by *E. histolytica* have never been done before. The present study, therefore, is the first to confirm the effectiveness of these drugs against experimental intestinal and hepatic amoebiasis of laboratory animals. These experimental results have direct bearing on the treatment of human cases of amoebiasis and confirm the presumption of these drugs to treat human amoebiasis patients.

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