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EVALUATION OF ANTICLOSTRIDIAL EFFICACY OF INDIGENOUS MEDICINAL PLANT DRUGS: RASOOT, AJWAIN KHURASANI, NEEM AND BAKAIN

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Studies were carried out to evaluate anticlostridial activity of Rasoot (crude aqueous extract of *Berberis aristata* seeds), Ajwain-khurasani *(Hyoscyamus niger* seeds), Neem *(Azadirachta indica* leaves) and Bakain *(Melia azedarach* leaves). The results of *in vitro* experiments have revealed that the aqueous extracts of these plant drugs do possess considerable antibacterial activity against *Clostridium perfringens* types B and D. The activity was comparable to standard discs of kanamycin and tetracycline. Preliminary *in vivo* evaluation has indicated that Rasoot possesses potency to treat enterotoxaemia in the goats. It is conceivable that these plant drugs contain some active principles which exert anticlostridial activity both *in vivo* and *in vitro* experiments.

INTRODUCTION

In folk medicine, many plants have been claimed to possess chemotherapeutic properties. Due to ever increasing problem of development of resistance to the conventional antibiotics, the interest in plants exhibiting antimicrobial activity has increased in recent years. Some of the research reports on the antibacterial activity of plant extracts include Malcolm and Sofowora (1969), Su *et al.* (1973), Afzal and Akhtar (1981) and Kumar and Sharma (1982). Certain extracts of plants are used to treat throat, chest and urinary tract infections (Ashraf and Bhatty, 1970). Thus, it is possible that some of the medicinal plants also possess anticlostridial property. Therefore, studies were carried out to evaluate the efficacy of Rasoot (crude water extracts of *B. aristata* seeds) Ajwain khurasani *(H. niger* seeds), Neem *(A. indica* leaves) and Bakain *(M. azedarach* leaves) against Clostridia which cause enterotoxaemia in goats.

MATERIALS AND METHODS

Plant material used: An aqueous extract of *B. aristata* locally called Rasoot and seeds of *H. niger* called Ajwain khurasani were purchased from a local herbal dealer. The fresh green leaves of *A. indica* (Neem) and *M. azedarach* (Bakain) were obtained from the botanical garden of the University of Agriculture, Faisalabad. These were dried under shade and were powdered with an electric grinder and stored in the well closed cellophane bags in a refrigerator.

Preparation of extracts: A 5% w/v aqueous solution of Rasoot was prepared by dissolving the solid materials in water for 72 hours and spun at 1500 rpm for 15 minutes and the

supernatant was used. To prepare aqueous extracts of seeds of Ajwain khurasani and leaves of Neem and Bakain, 100 g of each powdered drug was soaked and stirred well in 1000 ml of distilled water in stoppered glass containers and left for 24 hours. The contents were again stirred for about 10 minutes and the extract decanted and dried completely in petri dishes at 40 °C in a hot air oven. The dried powder was dissolved in water in the ratio of 5% w/v for further use.

also confirmed in rabbits (Shaddin *et al.*, 1979; Ijaz *et al.*, 1980).

Preparation of dilutions: Two-fold serial dilutions were prepared from the original aqueous extracts of plants in test tubes which were used for pouring onto the discs.

Preparation of discs: Impregnated paper discs method of Louis and Dean (1965) was employed. Discs of 5 mm diameter were cut from Whatman Filter Paper No. 1 and

Table 1. *In vitro* **anticlostridial activity of aqueous extracts of certain plants expressed as zones of inhibition***

Plant	Clostridial type	Dilutions			
		1:1	1:2	1:4	1:8
		Zones of inhibition (mm)			
Berberis aristata	B	18	17	17	-
	D	19	19	18	18
Hyoscyamus niger	B	17	17	16	-
	D	18	17	-	-
Azadirachta indica	B	16	16	16	-
	D	17	17	17	16
Melia azedarach	B	13	-	-	-
	D	15	-	-	-
Control	B	-	-	-	-
	D	-	-	-	-

*Mean of 3 observations.

Test organisms: *Clostridium perfringens* types B and D were isolated from the faecal samples of the goats suspected to be suffering from enterotoxaemia and confirmed by the microscopic, biochemical and serological tests. Their pathogenicity was

sterilised in hot air oven. A 0.02 ml of the original extract was dropped onto each disc and incubated at 37 °C for 30 minutes. The standard antibiotic discs of kanamycin, tetracycline and penicillin were used as control.

Anticlostridial Testing

a. ***In vitro*** **testing:** Sterilised cooked meat broth was anaerobically incubated separately with *Clostridium perfringens* types B and D at 37 ° C for 24 hours (Casals and Paderson, 1977). From every test tube, 0.1 ml of liquid broth culture of the organism was uniformly inoculated on the solid blood agar plates. Discs of test extracts alongwith the standard antibiotics (controls) were placed onto the blood agar plates previously seeded with bacteria.

b. ***In vivo*** **testing:** The goats found to be suffering from enterotoxaemia on the basis of the symptoms were divided into various groups of six each. These were treated with appropriate doses of the medicinal plant drugs or their extracts and with the standard antibiotics as detailed in Tables 3 and 4. The last group was kept as control without any treatment.

RESULTS AND DISCUSSION

Extracts of *B. aristata* (Rasoot) seeds, *H. niger* (Ajwain khurasani) seeds, *M. azedarach* (Bakain) seeds and *A. indica* (Neem) leaves have been empirically used in the folk medicine since centuries to treat various diseases of man and animals. In the present study, their aqueous extracts have showed considerable anticlostridial activity comparable to tetracycline and kanamycin. While control discs (without antibiotics) did not inhibit the growth of the Clostridia checked. The extracts of *B. aristata* and *A. indica* were more potent against *Clostridium perfringens* type D i.e. upto 1:8 dilution, while they showed activity upto 1:4 dilution against its type B. The *H. niger* extract was active against type B upto 1:4 dilution and less so against type D i.e. only 1:2 dilution produced a zone of inhibition. The *M. azedarach* extract produced a zone of inhibition against types B and D at 1:1 dilution only (Table 1).

Table 2. ***In vitro*** **anticlostridial activity of certain antibiotic discs expressed as zones of inhibition***

Antibiotics	Clostridial type	Zone of inhibition (mm)
Gentacin	B	27
	D	26
Kanamycin	B	22
	D	23
Tetracycline	B	18
	D	19
Penicillin	B	18
	D	19
Control	B	-
	D	-

*Mean of 3 observations.

It was observed that anticlostridial activity of these plant extracts was comparable to the standard antibiotics including kanamycin, tetracycline and penicillin. These findings are in agreement with those of Malcolm and Sofowora (1969) and Su *et al.* (1973) who reported such activities in certain other plant extracts. These results suggested that test indigenous medicinal plant extracts under study possessed marked anticlostridial activity under *in vitro* conditions.

The oral administration of the powdered plants and/or their extracts alongwith some antibiotics showed that 5 g/50 kg body weight of Rasoot exerted anticlostridial activity. Its tested dose showed 50% recovery

Table 3. *In vivo* **efficacy of medicinal plant drugs after their oral administration to the goats suffering from enterotoxaemia***

Plant drug	Dose/50 kg body weight (g)	Number of goats treated	Number of goats recovered	Number of goats died	Percentage of goats recovered
Aqueous extract of *B. aristata* seeds	5	6	3	3	50.00
Powdered *H. niger* seeds	250	6	2	4	33.30
Powdered *A. indica* leaves	250	6	1	5	16.60
Powdered *M. azedarach* leaves					

*Efficacy record after 48 hours on the basis of disappearance of symptoms.

Table 4. *In vivo* **efficacy of some antibiotics after parentral treatment of goats suffering from enterotoxaemia***

Drug	Dose (mg/kg b.w.)	Number of goats treated	Number of goats recovered	Number of goats died	Percentage of goats recovered
Gentacin	2-3 twice	10	8	2	80.00
Kanamycin	10 twice	7	4	3	57.10
Tetracycline	5 once daily	6	2	4	33.30
Penicillin	0.5	6	2	4	33.30

*Recovery judged after 48 hours on the basis of disappearance of symptoms.

while kanamycin injected group produced 57% recovery. The control group showed 100% mortality. It is, therefore, conceivable that if its dose is increased, it may become more potent. Thus Rasoot is a promising herbal drug which needs further investigation. Therefore, further studies are suggested to prove its real worth as chemotherapeutic agent for Clostridial enterotoxaemia in the goats.

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