

AN EXPERIMENTAL STUDY OF KUTAJARISHTA WITH SPECIAL REFERENCE TO AMOEBIASIS

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ABSTRACT: A study on Kutajarista' was carried out and it was tested for anti-amoebic action which is presented in this paper.

Kutajarista is self generated alcohol containing ayurvedic preparation. It is extensively being used in disorders like Atisar and Pravahika by Ayurvedic physicians. 'Kutaja', the main ingredient of Kutajarista is a well known remedy of Ayurveda, as its name (Holarrhena anti-dysenterica) indicated for the treatment of diarrhea and dysentery. Keeping it is view an experimental study was carried out, in collaboration with the Department of Microbiology, Institute of Medical Sciences to verify the above claims and also to see its effect on *E. histolytica* in vitro and in vivo. For this purpose we have prepared 3 different samples of the Kutajarista (Kutajarista A,B,C). Out of the 3 samples A & B are prepared by following the Arista method while C was prepared by following the Asava method.

The drugs were tested for antiamoebic action in vitro cultures of *E-histolytica*. The drugs were added in graded concentration

and control cultures with equivalent amount of alcohol present in the drugs were examined after 48 hours incubation.

The drugs did not show any action of *E. histolytica* cultures as was evident from the number of amoebae in drug containing media and control tubes.

Many drugs may be inactive in vitro against an organism but some metabolic product of them may be effective. To find out any break down product in vivo of the drugs namely Kutajarista has any effect on *E. histolytica*, experimental studies in animal were undertaken. In addition a few drugs that may not have action on the infective agent, may give relief of symptoms in patients, due to their action of the inflammatory process involved in the disease.

Materials and Methods

Experimental study on the effect of 'Kutajarista' on amoebiasis was under taken in guineapig of about 70 to 80 gms weight. The animals were obtained from the Central Animal House of the Institute of Medical Sciences.

E. histolytica was isolated from faeces of a case of dysentery by culture in modified Boek and Drbohlav's medium. The culture was maintained in the laboratory by serial subculture in the above medium at intervals of 72 hours. For inoculation in animals the strain of *E. histolytica* was cultured in several tubes of the medium and incubated for 48 hours. At the end of incubation the fluid phase of the cultures pooled, centrifuged at 1000xg for 10 minutes. The supernatant was discarded and the deposit was washed three times with phosphate buffer saline (pH 7.2). Finally the deposit was suspended in small volume of phosphate buffer saline, the number of amoebae per ml of the suspension was ascertained by counting in a modified New-berg's Chamber. The count of amoebae was adjusted to 2×10^5 per ml by adding required amount of phosphate buffer saline. 0.5 ml of this suspension containing 10^5 amoebae was used for infecting each animal.

The guineapigs were kept without food for one day before inoculation but were given adequate water. On the day of inoculation each animal was anaesthetized by anaesthetic ether. A paramedian incision on the right of the amoeba suspension was injected into it, using a 20 number hypodermic needle and a tuberculine syringe. The abdominal opening was sutured by interrupted suture using silk thread. After recovery from

anaesthetic effect the animals were divided into 4 groups; one being control, the other 3 were given treatment as detailed below:

The drugs were started from 48 hours after inoculation. 0.5 ml each drug was administered orally with the help of a fine polythene tube and a tuberculine syringe. The drug was given orally, once a day for 5 days. The animals were sacrificed on the 7th day. Before sacrificing under ether anaesthesia blood was collected from the heart of the animals. The caecum was taken out from each animal and the caecal pathology was assessed according to the criteria of Neal (1951).

Criteria for scoring

Wall

Normal	0
Slight thickening	1
Marked local thickening and contraction with slight hyperaemia	2
Extensive thickening and contraction and gross hyperaemia.	
Ulcer may or may not be seen	3
Caecum shapeless with extensive thickening, gross contraction,	
Many ulceration and severe hyperaemia	4

Content

Normal and shows no trophozoite by microscopic examination	0
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Slightly less solid than normal, microscopical examination shows 1 to 10 trophozoites per low power field

Slightly mucoid, microscopically pus cells may be seen, number of trophozoites vary between 20 to 40 per low power field

Mucoid, with little matter, plenty of pus cells, trophozoites vary between 60 to 80 or more per low power field

No solid matter, blood and mucus, microscopically full of pus cells and RBC with plenty of trophozoites 4

The virulence of *E-histolytica* strains was expressed in the terms of caecal score which was sum of the two values obtained by scoring the condition of the wall and the caecal content separately. Maximum score possible was 8.

The blood collected from the animals was immediately mixed with heparin and processed for estimation of serotonin and histamine.

Observations

Caecal scores as noted were as follows:

Control group	4
Treated groups:	
A	3.5
B	4
C	3

Result of serotonin Estimation

Control 0.58 microgram/ml.

Group:

A	0.24	“	“
B	0.22	“	“
C	0.26	“	“

Discussion

When the drug was given to patients with amoebiasis there was symptomatic relief. So it was necessary to find out whether the drugs have only action on the amoebae when the drugs were screened for anti-amoebic action in vitro, it was found that they do not inhibit the growth of the amoeba in culture.

In animal experiment the drugs did not show any significant action on the caecal pathology. These above findings do not correlate with the observation of symptomatic relief in clinical amoebiasis.

The notable finding of the action of the drugs was significantly lowering the level of 5-hydroxytryptamine (serotonin) in the blood of treated animals. It has been reported earlier that lowering of peripheral level of 5-hydroxytryptamine in the host, reduces the disease producing capacity of *E.histolytica* (Entiaz Ahmad et al., 1984). It is possible that the drug in question do not have any direct action on *E-histolytica*. But by lowering the peripheral serotonin level in patients the drug may reduce the virulence of the amoeba and thereby providing symptomatic relief to the patients. For the study in this direction is needed to definitely establish the above possible action of the drug in amelioration of amoebiasis.

Conclusion

Thus it can be concluded that the drug 'Kutajarista' lowers the peripheral level of 5-hydroxytryptamine thereby helps in decreasing the virulence of E-histolytica. The 3 samples of E-histolytica. The 3 samples of Kutajarista have more or less same effects. It has got no amoebicidal action invivo or is vitro.

Selected Bibliography

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