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A COMPARATIVE STUDY ON MUSTAKARANJADI KWATHA AND MUSTAKARANJADI ARISHTA W.S.R TO THEIR ANTI BACTERIAL ACTIVITY IN DIARRHEA

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ABSTRACT

Diarrhoea is a disease characterized by the frequency of loose and watery stools and this mimic, one of the symptoms mentioned under the heading of Atisara in Ayurvedic classics. Mustakaranjadi kwatha is one of the famous formulations mentioned in Sahasrayoga which is indicated for atisara. A new dosage form is also formulated from the same yoga, i.e Mustakaranajdi arishta and both the formulations are analysed for its anti bacterial effect. In the Anti bacterial study, zone of inhibition of both the samples were tested against three bacteria namely *Salmonella typhimurium, Shigella flexneri, Escherichia coli.* The results were then compared and analysed. The zone of inhibition was not found against three organisms in Mustakaranjadi kwatha while Mustakaranajadi arishta found anti bacterial effect on E.coli.

KEY WORDS: Diarrhoea, Mustakaranjadi kwatha, atisara.

INTRODUCTION

A ‘Drug’ is a fundamental patient management tool in the hands of a physician according to Ayurvedic classics. The information related to the drugs and formulations along with the diagnosis and management of disease guided with techniques of health maintenance through observation of proper daily and seasonal routines can be found in various classical and literary works.

Acharya Charaka formulated the word kalpa for pharmaceutical preparations. The chakrapani commentator of Charaka defines the kalpana as *shakti visesham kalpanardham cha kalpanam.*[1] which indicates that kalpana should have vishesha shakti. One can convert the drug into different dosage forms by giving different samskaras. Samskara is a technique, which turns raw drugs into potent medicine. Acharya Charaka describes that even the mixing of drugs causes some changes in properties and that also should be considered as samskara.[2] Therefore to attain the bioavailability of a particular formulation it should strictly undergo the procedures of samskara.

Diarrhoea is the 3rd leading cause of childhood mortality in India and is responsible for 13% of all deaths / year in Children under 5 years of age. It acts as a symptom as well as a disease entity. From the Ayurvedic classics Atisara can be considered as diarrhoea having the same symptom of *atidrava mala pravrti*, which is caused by multifactorial nidanas like *Virudha ahara, visha, bhaya, shoka, dushtambu sevana, krimi* etc. Diarrhoea is mostly caused by bacterial, viral or parasitic infection of the bowels. The response of these bacteria to our Ayurvedic classics if studied, will definitely contribute to the proper management of atisara. Mustakaranjaadi kwatha[3] is one of the many medicines quoted in Atisara chikitsa mentioned in Sahasrayoga.

Hence this study is undertaken to understand the action of Mustakaranjadi kwatha on the causative organisms of diarrhoea such as Eshcherichia coli, Salmonella typhimurium and Shigella flexneri and also to formulate a new dosage form ie Arishta for better palatability and to increase shelf life. Therefore this study is done to compare the effect of both Mustakaranjadi kwatha and Mustakaranjadi arishta in the anti bacterial activity of Diarrhoea.

AIMS AND OBJECTIVES

To evaluate the anti bacterial activity of MKK and MKA on Escherichia coli, Salmonella typhimurium, Shigella flexneri.

Source of data

The evaluation of antibacterial activity of MKK and MKA was done at S.D.M Centre for Research and Allied sciences, Udupi.

Antibacterial study of Mustakarnjadi kwatha (MKK)

To evaluate the antibacterial activity of Mustakaranjadi kwatha on *Escherichia coli, Salmonella typhimurium, Shigella flexneri* by well diffusion method.

MATERIALS REQUIRED

- Test strain- E. coli, Salmonella typhimurium, Shigella flexneri.
- Distilled water, saline.
- Test tube, Incubator, Laminar air flow.
- Graduated micropipettes.
- Growth medium- Nutrient agar.
- Sample – Mustakaranjadi kwatha.

METHOD

Preparation of Nutrient agar media:[4]

Beef extract (1g), yeast extract (2g), peptone (5g) and Sodium Chloride (5g) were dissolved in 990ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000ml. Finally 15g agar was added to the media and autoclaved to 121^{o}C for 20 minutes.

PREPARATION OF INOCULUMS

All the 3 bacteriae were procured from culture collection centre, IMTECH, Chandigarh. Loopful of 48h old culture from the slants was transferred to sterile saline and mixed well to prepare homogenous inoculums.

WELL DIFFUSION METHOD[5]

The media was cooled to around 45-55^{o}C, around 20ml each was poured into sterile petriplates. One ml of the each inoculums were immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plated were incubated overnight at 37^{o}C and observed after 48hours.

Samples of mustakaranjadi kwatha taken - 10µl, 25µl, 50µl.

Control (Distilled water) - 30µl, Standard (Ampicillin) - 30µg.

Antibacterial activity of Mustakaranajdi arishta(MKA)

Here tube method was followed.

To evaluate the antibacterial activity of Mustakaranajadi arishta on *Escherichia Coli, Salmonella typhimurium, shigella flexneri.*

Materials required

- Test strain – Eshcherichia Coli.
- Sterile saline.
- Test tubes, Microfuge tubes, Incubator.
- Hot air oven, Laminar air flow.
- Growth medium – Nutrient agar medium.
- Sample- MKA.

METHOD

The Nutrient agar medium and inoculums are prepared same as mentioned above.

Sample preparation[6]

Zero to 100µl of arishta is diluted with 2ml of medium and the suspension is inoculated. After overnight incubation the suspension was transferred to pre weighed microfuge tube, centrifuged at 10000 rpm. The pellet was washed twice with sterile saline and the wet weight of the pellet obtained from arishta treated sample (E.coli - 40µml, salmonella - 30µml, Shigella - 30µml) and the control suspension were subjected to serial dilutions and plated on to nutrient agar medium. After overnight incubation the colonies were counted.

Number of dilutions taken for control group sample in E. coli- 10^3, 10^5, 10^6, 10^7, 10^{10}.
Number of dilutions taken for arishta treated samples in E. coli – 10^3, 10^5, 10^6, 10^7, 10^{10}.
Number of dilutions taken for control in S. Typhimurium – 10^3, 10^5, 10^6, 10^7, 10^{10}.
Number of dilutions taken for arishta treated sample in S. Typhimurium – 10^3, 10^5, 10^6, 10^7, 10^{10}.
Number of dilutions taken for control in Shigella flexneri – 10^3, 10^5, 10^6, 10^7, 10^8.
Number of dilutions taken for arishta treated samples in Shigella flexneri- 10^3, 10^5, 10^6, 10^7, 10^8, 10^{10}.

RESULTS

Table 1: Zone of Inhibition of MKK against E. coli.

Sample	Zone of inhibition (Radius in mm)
10µl	0
25µl	0
50µl	0
Control (distilled water) 30µl	0
Standard (ampicillin) 30µg	10

Table 2: Zone of inhibition of MKK against S. Typhimurium.

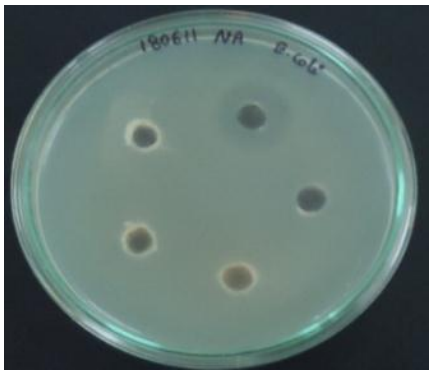
Sample	Zone of inhibition (Radius in mm)
10µl	0
25µl	0
50µl	0
Control (distilled water) 30µl	0
Standard (Ampicillin) 30µg	13

Table 3: Zone of inhibition of MKK against Shigella flexneri.

Sample	Zone of inhibition (Radius in mm)
10µl	0
25µl	0
50µl	0
Control (Distilled water) 30µl	0
Standard (Ampicillin) 30µg	13

Conclusion – The tested sample did not show antibacterial activity against E.coli, S. typhimurium, shigella flexneri.

Antibacterial study of Mustakaranajdi kwatha



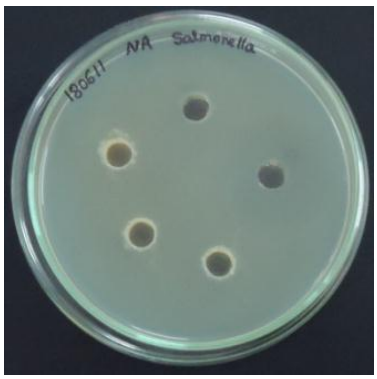


Fig 1: Antibacterial effect on E.coli. **Fig 2: Antibacterial effect on S.typhimurium.**

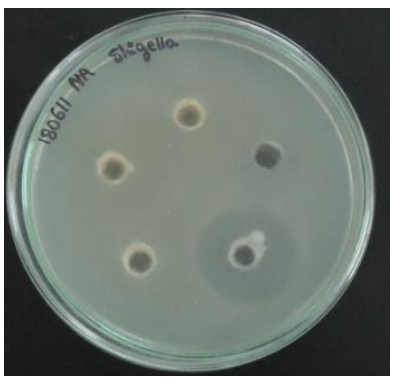


Fig 3: Antibacterial effect on Shigella flexneri.

2 **Antibacterial study on Mustakaranajdi arishta**

Sample solutions of Mustakaranjadi arishta in E.coli.

Fig 4. 10^5.

Fig 5. 10^6.

Fig 6. 10^7.

Fig 7. 10^8.

Sample solutions of mustakaranajdi arishta in salmonella typhimurium.

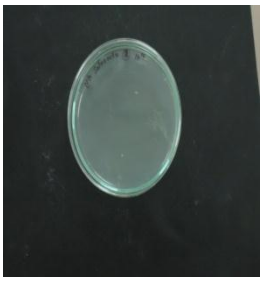


Fig 8. 10^{10}.

Fig 9.10^6.

Fig 10.10^7.

Sample solutions of mustakaranajdi arishta in shigella flexneri.

Fig 11.10^8.

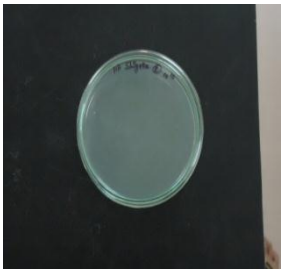


Fig 12. 10^{10}.

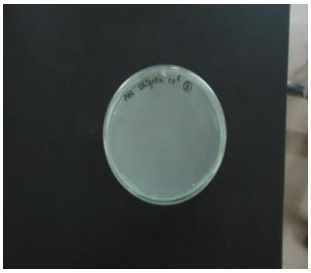


Fig13.10^6.

In vitro antibacterial activity of MKA on E.coli.

Control: Table 4: Number of colonies of Control group of E.coli.

Sl. No.	Dilutions	Number of colonies (NOC)	CFU/ml
1	10^3	Mat growth	Mat growth
2	10^5	Mat growth	Mat growth
3	10^6	High	High
4	10^7	high	High
5	10^8	350	3.5 x 10^{10}

With arishta

Table 5: Number of colonies of arishta treated group of E.coli.

Sl. No	Dilutions	Number of colonies(NOC)	CFU/ml
1	10^3	Mat growth	Mat growth
2	10^5	High	High
3	10^6	High	High
4	10^7	280	2.8×10^9
5	10^8	10	1.0×10^9

Conlusion: In case of E.coli arishta was found inhibitory effect. It is found that the total count of the organism was 3.5×10^{10}/ml in untreated sample where as in Arishta treated sample it was around 2.8×10^9/ml. Thus around 3 fold decreases in E.coli count was seen due to arishta.

In vitro antibacterial effect of MKA on Salmonella typhimurium

Control: Table 6: Number of colonies of Control group of Salmonella typhimurium.

Sl No	Dilutions	Number of colonies (NOC)	CFU/ml
1	10^3	High	High
2	10^5	190	1.9×10^7
3	10^6	23	2.3×10^7
4	10^7	7	7.0×10^7
5	10^{10}	10	1.0×10^{11}

With arishta

Table 7: Number of colonies Arishta treated group of Salmonella typhimurium.

Sl No	Dilutions	Number of colonies (NOC)	CFU/ml
1	10^3	Mat growth	Mat growth
2	10^5	Mat growth	Mat growth
3	10^6	Mat growth	Mat growth
4	10^7	Mat growth	Mat growth
5	10^{10}	80	8.0×10^{11}

Conclusion: In case of Salmonella typhimurium arishta did not exhibit inhibitory effect

In vitro anti bacterial effect of MKA on Shigella flexneri

Control: Table 7: Number of colonies Control group of Shigella flexneri.

Sl.No	Dilutions	Number of colonies (NOC)	CFU/gm
1	10^3	Mat growth	Mat growth
2	10^5	Mat growth	Mat growth
3	10^6	Mat growth	Mat growth
4	10^7	High	High
5	10^8	18	1.8×10^9

With Arishta:

Table 8: Number of colonies Arishta treated group of Shigella flexneri.

Sl No	Dilutions	Number of colonies (NOC)	CFU/ml
1	10^3	Mat growth	Mat growth
2	10^5	Mat growth	Mat growth
3	10^6	Mat growth	Mat growth
4	10^7	Mat growth	Mat growth
5	10^8	33	3.3×10^9
6	10^{10}	6	6.0×10^{10}

Conclusion: In case of Shigella Flexneri Arishta did not exhibit inhibitory effect. The figures are given below. (Fig 4,5,6,7,8,9,10,11,12,13).

DISCUSSION

An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi, protozoans etc. Antimicrobials are classified into two broad categories on the basis of mode of action. a. Microbicidal that kills microbes without leaving any option for their survival. b. Microbistatic that cease all the metabolic activities of microbes that are important for their survival and so they are called as growth inhibitors of microbes.

Mustakaranjadi kwatha is a formulation mentioned in Sahasrayoga for the treatment of atisara. Here a new dosage form is also designed with the same yoga ie mustakaranjadi arishta. This study was carried out with an objective to investigate the antibacterial effect of MKK and MKA against diarrhoea causing organisms. The aim of the study is to assess the zone of inhibition of the two samples and to compare the results on bacterias like Escherichia coli, Salmonella typhimurium, Shigella flexneri.

The zone of inhibition was not found in any of the organisms treated with MKK. The pharmacological action of the drug inside the body may be supported by some other factors like immunologically or cellularly, and may be due to this there is no effect for MKK directly on the diarrhoea causing organisms.

Kashaya was lighter in colour while arishta was dark brown. However the dark brown colour of arishta diffused around the well and hence the result could not be inferred. Therefore tube method was followed to study the antibacterial effect of arishta. In arishta treated samples only E.coli found inhibitory effect. Around 3 fold decrease in E.coli count was seen due to arishta. The other two organisms didn't show any inhibitory effect. Due to the fermentation process the possibility of antimicrobial product may form which is possible to inhibit E.coli

and may be due to this it shown effect on E.coli. The negative microbiological result does not establish that there is no clinical efficacy for Mustakaranjadi yoga. However the clinical effectiveness of this formulation throws light on the fact that there are other unexplored factors which contribute to its efficacy.

CONCLUSION

Mustakaranjadi kwatha is a formulation which is widely used for atisara. Even though it is clinically used the antibacterial activity against diarrhoea causing organisms was not known. Therefore this study was undertaken to understand its antibacterial activity and also to design a new dosage form ie mustakaranjadi arishta and to know its antibacterial activity and compare the results of both.

This study shows that Mustakaranjadi kwatha did not found any zone of inhibition against diarrhoea causing organisms E.coli, Salmonella typhimurium and Shigella flexneri. Mustakaranjadi arishta exhibited antibacterial activity only on E.coli.

The negative microbiological result does not establish that there is no clinical efficacy for mustakaranjadi yoga. However the clinical effectiveness of this formulation throws light on the fact that there are other unexplored factors which contribute to its efficacy.

REFERENCES

1. Agnivesha, Charaka Samhita, revised by Charaka and Dhridhabala with the Ayurveda – Dipika commentary of Chakrapanidatta edited by Vaidya Jadavji Trikamji Acharya, 1st Ed. Varanasi: Chaukamba Surabharathi prakashan, 2016; 31: 738.
2. Agnivesha, Charaka Samhita, revised by Charaka and Dhridhabala with the Ayurveda – Dipika commentary of Chakrapanidatta edited by Vaidya Yadavji Trikamji Acharya, 1st Ed. Varanasi: Chaukamba Surabharathi prakashan, 2016; 13: 738.
3. Abhinava Vaghbhatan, Sahasrayogam with Sujanapriya Vyakhya edited by K.V.Krishnan Vaidyan & S.Gopala Pillai, 26th Ed. Mullakal: Vidyarambham Publishers, 2006; 43: 544.
4. Cleidson Valgas, Simone machado de Souza, Elza F A Smania, Artur Smania Jr; Screening methods to determine antibacterial activity of natural products; Brazilian Journal of Microbiology, 2007; 38: 369-380.
5. Mohammed K. Almaghrabi, Antimocrobial activity of Salvadora Persica on Streptococcus Pneumoniae; Biomedical Research, 2018; 29(19): 3635-3637.

6. Shigeharu, Yumi Fukui, Masaya Yamamoto, Yuki Masuda, Akira Kamiya; In vitro antimicrobial effects of aztreonam, colistin, and the 3 drug combination of aztreonam, ceftazidime and amikacin on metallo-b-lactamase- producing Pseudomonas aeruginosa; BMC Infectious Diseases, 2009; 9: 123.