Research Article

www.ijrap.net (ISSN:2229–3566)

EFFICACY OF CHAKRAMARDA PATRA SWARASA (*CASSIA TORA* LINN) AND DADHI IN KRIMIJA ATISARAM: A PRECLINICAL APPROACH

Sreelekshmi S *[1], Priya S. [2], Priyalatha B [3]

[1] Assistant Professor, KMCT Ayurveda Medical College, Kozhikode, Kerala, India

[2] Professor, Department of Dravya guna (Ayurvedic Pharmacology), Amrita School of Ayurveda, Amrita Vishwa Vidhyapeetham, Kollam, Kerala, India

[3] Associate Professor, Department of Dravya guna (Ayurvedic Pharmacology), Amrita School of Ayurveda, Amrita Vishwa Vidhyapeetham, Kollam, Kerala, India

Received on: 19/08/20 Accepted on: 11/09/20

***Corresponding author**
E-mail: sl301191@gmail.com

DOI: 10.7897/2277-4343.1105171

ABSTRACT

Diarrhoea is a major public health problem especially in developing countries. The signs and symptoms of Diarrhoea can nearly be co-related to Atisara. Diarrhoea caused by bacterial pathogens can be equated with the lakshanas seen in Krimija Atisara. Hetu vyadhi pratyanika chikitsa is explained by Acharya Sushruta for treating Krimija Atisara. In Yogamritam Atisara chikitsa Adhikara; it is explained that Chakramarda patra Swarasa, taken along with Dadhi is effective in Atisara. Hence, the study has been undertaken as a pre-clinical step- *In vitro* Agar well diffusion model, Microorganisms used for the study are *E coli* and *Staphylococcus aureus*. Two trial groups were included in this study; trial group 1- Chakramarda patra Swarasa alone, Trial group 2- Chakramarda patra Swarasa with Dadhi. Gentamicin was used as Standard. It was observed that the 2 trial groups showed Zone of inhibition against *E coli* and *Staphylococcus aureus*. Both the trial groups showed More Zone of inhibition against *Staphylococcus aureus* than *E coli*. Mean zone of inhibition of the 2 trial groups was found to be lower as compared to that of Mean zone of inhibition of the standard against *E coli* and *Staphylococcus aureus*. Student 't' test is used to analyze the data. The 't' value is not statistically significant at the level of $P < 0.05$. Hence null hypothesis is accepted and alternate hypothesis is rejected.This article aims to give a scientific validity to usage of Chakramarda patra Swarasa with Dadhi in Krimija Atisara.

Keywords: Chakramarda, Dadhi, Gentamicin, Krimija Atisara, Agar well diffusion, *E coli, Staphylococcus aureus*

INTRODUCTION

The emergence of multidrug resistant bacterial and fungal strains has increased substantially in the recent years. It is a serious problem in the medical field worldwide. The main reason behind this problem is excess and uncontrolled use of antibiotics. By this Mutation happened in the pathogens which are responsible for the formation of antibiotic resistant pathogens. World Health organization made effort to encourage the development of effective herbal origin antimicrobial drugs, which are safe, effective, with less or no side effects. Researchers stated that plant extracts show target sites other than those used by antibiotics, which will be active against drug resistant pathogens. This study has been undertaken as a pre-clinical step, considering the greater prevalence of the disease Diarrhoea and need for the search of a cost-effective herbal origin antimicrobial medicine that can prevail bacterial diarrhea.

The signs and symptoms of diarrhea can nearly be co-related to Atisara. In Sushruta Samhita Uttara tantra, Atisara Pratishedha Adhyayam, Acharya described about Krimija Atisāram[1]. The lakshana of Krimija Atisaram should be understood according to the dosha dushti lakshana shown by the patient. Acharya explained Hetu vyadhi viparita chikitsa for the management of such condition. In Yogamritam Atisara chikitsa Adhikaram[2], it is explained that Chakramarda patra Swarasa, taken along with Dadhi is effective in Atisara. Literature search of Chakramarda and Dadhi clarified hetu vyadhi Pratyanika action of Chakramarda and Dadhi on Krimija Atisaram. Hence, this *in vitro* study had been done, as a preclinical step, to give scientific validation of this Yogam.

MATERIAL AND METHODS

The present study was carried out fewer than three headings:

1. Collection and preservation of the drugs
2. Preparation of Medicine
3. *In-vitro* Study

Study drug

The drug used in the study was Chakramarda Patra Swarasa (Leaf juice of *Cassia tora* Linn) and Dadhi.

Collection and Preservation of the drugs

Study drug was collected from Amrita herbal garden during the month of December in the year 2017 and were taxonomically identified. Leaves which are not too tender and not too older were collected for the study. Homemade curd was used for study.

Preparation of medicine

Preparation of Chakramarda patra Swarasa

The collected leaves of Chakramarda were washed thoroughly, removed all earthy and foreign materials and then Swarasa was taken out, by crushing the leaves with the help of mortar and pestle. Squeeze the crushed leaves and obtained Swarasa was properly strained through a clean cloth.

Figure 1: Cassia tora[3]

Preparation of Dadhi

Curd was prepared from boiled cow's milk. Milk was boiled and then it was allowed to cool (bring down the temperature into room temperature). After cooling, an inoculum, as starter culture was introduced (stale curd).

The starter culture mainly composed of *Lacto bacillus* bacteria. The preparation was then kept at room temperature (25-35°C), for 3-5 hours in summer and overnight during winter.[4]

Methods

The methodology of the study was divided into 2 sections

Pharmacognostical analysis

Pharmacognostical and Physico chemical analysis of Chakramarda Patra (Leaf of *Cassia tora* Linn) was carried out in Department of Dravya Guṇa Vijnana and Quality control lab of Amrita School of Āyurvēda. Morphological, histological and powder microscopic peculiarity of *Cassia tora* was noted.[5]

In-vitro study

In-vitro study refers to the technique of performing a given procedure in a controlled environment outside of a living organism.[6]

Media Used

Mueller-Hinton Agar (Himedia)

Microorganisms Used

Escherichia coli (NCIM 2065), *Staphylococcus aureus* (NCIM2127)

Samples to be tested

Chakramarda patra Swarasa (Leaf juice of *Cassia tora* Linn)

Combination of Chakramarda patra Swarasa and Dadhi (Curd) (1:1 ratio)

Standard: Gentamicin (0.1 ml of Gentamicin is diluted with 9.9 ml distilled water, from that 100 mcl is taken)

Media preparation and antimicrobial analysis

The bacteria procured from Care Keralam were sub-cultured and it was used for the study. The nutrient broth was prepared and a colony of bacteria from the culture was inoculated to it and kept for incubation.

Preparation of Muller –Hinton agar

3.8 g of Mueller-Hinton Agar was dissolved in 100 ml distilled water; sterilized by autoclaving at 121°C for 15 minutes at 15 lbs pressure. After sterilization, media was cooled to 45°C in water bath. Then 25 ml media was poured into sterile Petri-plates and allowed to solidify.

After that, 3 hours old bacterial culture was swabbed on the surface of MHA plates and kept for drying the inoculum. Then, using sterile cork borer, wells were prepared on the agar media, different cork borers should be used for different organism. 150 mcl, 100 mcl, 5o mcl samples of Chakramarda patra Swarasa and Chakramarda patra Swarasa with Dadhi were added to the well by using micro pipette. Gentamicin was kept as Standard drug (0.1 ml Gentamicin diluted with 9.9 ml distilled water, made it as 10 ml, from that 100 mcl is taken). After the complete diffusion of sample into the agar well, the plates were kept for incubation at 37°C for 24 hours. After 24 hours, Zone of Inhibition was measured using Scale/ Vernier caliper. Obtained results were analyzed with the help of Measures of central tendency-Arithmetic mean and Student 't' test.

RESULTS

Macroscopic evaluation of *Cassia tora* Leaf

Size - About 7.5-10 cm long
Shape - Obovate, Oblong, glaucous, membranous, glabrous or more or less pubescent, base somewhat oblique, usually rounded.
Colour - Green
Odour - Characteristic odour
Taste - Bitter

***Cassia tora* leaf powder microscopy**

Organoleptic Evaluation of *Cassia tora* Leaf Powder
Colour - Dark green
Odour - Characteristic strong penetrating odour
Fracture - Coarse powder
Taste - Faint astringent taste

Powder shows Stomatal fragments, covering trichome, vascular strand, fiber, calcium oxalate crystals, Starch grains.

Zone of Inhibition shown by the two samples and standard Gentamicin against *E. coli* and *Staphylococcus aureus*[7].

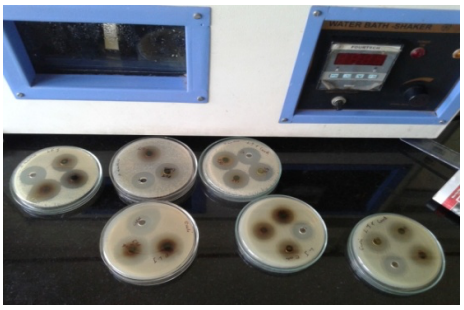

Figure 2: ZOI against *E. coli*

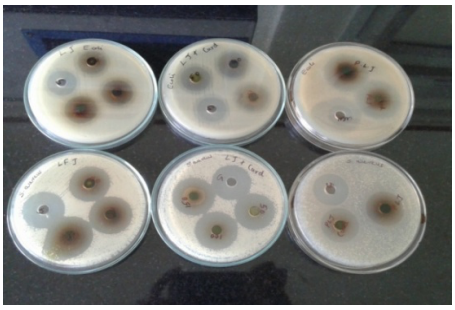

Figure 3: ZOI against *Staphylococcus aureus*

Table 1: Zone of inhibition of 2 trial groups and standard against *E. coli*

Parameters	Sample name	Result Zone of inhibition (Diameter in mm)			Control Zone of inhibition (Diameter in mm) Gentamicin
		50 mcl	100 mcl	150 mcl	100 mcl
E coli NCIM No:2256	Chakramarda patra Swarasa				
	1	9	10.5	12	22
	2	10	11.5	12	22
	3	9.5	11	11	23
	4	11	12	12	22
	5	10	11	11.5	23
	Chakramarda patra Swarasa and Dadhi				
	1	10	11.5	12	23
	2	11	12	12	22
	3	9	10.5	11.5	23
	4	11.5	12	13	22
	5	10	11	12.5	23

Table 2: Zone of inhibition shown by 2 trial groups and standard against *Staphylococcus aureus*

Parameters	Sample name	Result Zone of inhibition (Diameter in mm)			Control Zone of inhibition (Diameter in mm) Gentamicin
		50 mcl	100 mcl	150 mcl	100 mcl
Staphylococcus aureus NCIM No:2127	Chakramarda patra Swarasa				
	1	14	14	15	26
	2	13.5	15	16	27
	3	14	14.5	15	28
	4	13	15	15.5	27
	5	12.5	14	16	26
	Chakramarda patra Swarasa and Dadhi				
	1	13	15	14.5	27
	2	14	14.5	15	28
	3	14	16	15.5	26
	4	13	15	16	27
	5	13	14.5	15	26

Table 3: Mean zone of inhibition shown by 2 trial groups and standard against *E. coli*

Bacteria	Group	Mean Zone of Inhibition (in mm)
E coli	**Group 1**	
	1.Chakramarda patra Swarasa 50 mcl	9.9
	2. Chakramarda patra Swarasa 100 mcl	11.2
	3. Chakramarda patra Swarasa 150 mcl	11.7
	4. Gentamicin 100 mcl	22.4
	Group 2	
	1. Chakramarda patra Swarasa and Dadhi 50 mcl	10.3
	2. Chakramarda patra Swarasa and Dadhi 100 mcl	11.4
	3. Chakramarda patra Swarasa and Dadhi 150 mcl	12.2
	4. Gentamicin 100 mcl	22.6

Table 4: Mean zone of inhibition shown by 2 trial groups and standard against *Staphylococcus aureus*

Bacteria	Group	Mean Zone of Inhibition (in mm)
Staphylococcus aureus	**Group 1**	
	1. Chakramarda patra Swarasa 50 mcl	13.4
	2. Chakramarda patra Swarasa 100 mcl	14.5
	3. Chakramarda patra Swarasa 150 mcl	15.5
	4. Gentamicin 100 mcl	26.8
	Group 2	
	1. Chakramarda patra Swarasa and Dadhi 50 mcl	13.4
	2. Chakramarda patra Swarasa and Dadhi 100 mcl	15
	3. Chakramarda patra Swarasa and Dadhi 150 mcl	15.2
	4. Gentamicin 100 mcl	26.8

Statistical analysis of obtained results

Results were statistically analyzed by Student 't' Test

Table 5: ZOI shown by the samples against *E. coli* Bacteria in 50 mcl concentration

S. No	ZOI in Chakramarda patra Swarasa 100 mcl	ZOI in Chakramarda patra Swarasa and Dadhi 100 mcl	t Value	Statistically significant or not
1	10.5	11.5	0.516	Not Significant At the level p < 0.05 (5% level)
2	11.5	12		
3	11	10.5		
4	12	12		
5	11	11		

Table 6: ZOI shown by the samples against *E. coli* Bacteria in 100 mcl concentration

S. No.	ZOI in Chakramarda patra Swarasa 50 mcl	ZOI in Chakramarda patra Swarasa + Dadhi 50 mcl	t Value	Statistically significant or not
1	9	10	0.730	Not Significant At the level p < 0.05 (5% level)
2	10	11		
3	9.5	9		
5	11	11.5		

Table 7: ZOI shown by the samples against *E. coli* Bacteria in 150 mcl concentration

S. No.	ZOI in Chakramarda patra Swarasa 150 mcl	ZOI in Chakramarda patra Swarasa and Dadhi 150 mcl	t Value	Statistically significant or not
1	12	12	1.543	Not Significant At the level p < 0.05 (5% level)
2	12	12		
3	11	11.5		
4	12	13		
5	11.5	12.5		

Table 8: ZOI shown by the samples against *Staphylococcus aureus* Bacteria in 50 mcl concentration

S. No.	ZOI in Chakramarda patra Swarasa 50 mcl	ZOI in Chakramarda patra Swarasa and Dadhi 50 mcl	t Value	Statistically significant or not
1	14	13	0	Not Significant At the level p < 0.05 (5% level)
2	13.5	14		
3	14	14		
4	13	13		
5	12.5	13		

Table 9: ZOI shown by the samples against *Staphylococcus aureus* Bacteria in 100 mcl concentration

S. No.	ZOI in Chakramarda Swarasa 100 mcl	ZOI in Chakramarda patra Swarasa and Dadhi 100 mcl	t Value	Statistically significant or not
1	14	15	1.414	Not Significant At the level p < 0.05 (5% level)
2	15	14.5		
3	14.5	16		
4	15	15		
5	14	14.5		

Table 10: ZOI shown by the samples against *Staphylococcus aureus* Bacteria in 150 mcl concentration

S. No.	ZOI in Chakramarda patra Swarasa 150 mcl	ZOI in Chakramarda patra Swarasa and Dadhi 150 mcl	t Value	Statistically significant or not
1	15	14.5	0.395	Not Significant At the level p < 0.05(5% level)
2	16	15		
3	15	15.5		
4	15.5	16		
5	16	15		

DISCUSSION

To establish the quality and reliability of the drugs, various Pharmacognostical screening measures were adopted. The procedures were carried out at Quality control lab of Amrita School of Āyurveda. The macroscopic and microscopic features of the collected sample drug were compared with that of the description of Chakramarda patra (*Cassia tora* Linn) available in Āyurveda Pharmacopoeia of India and other authentic Pharmacognostic texts.

In-vitro study has been designed to evaluate comparative antimicrobial activity of Chakramarda patra Swarasa and Dadhi in selected pathogens causing Diarrhoea such as *E. coli* and *Staphylococcus aureus*. The bacteria were procured from care Keralam, Koratty. Study was done in aseptic conditions.

Zone of inhibition formed around the well was the criteria of assessment. Zone of inhibition were measured after 24-hour incubation. The Diameter of zone of inhibition showed by the samples against each bacterium was measured using scale in mm. It was observed that both trial groups and Standard showed zone of inhibition against *E. coli* and *Staphylococcus aureus*.

Both the trial groups showed More Zone of inhibition against *Staphylococcus aureus* than *E. coli*. This is because, *E. coli* is a gram-negative bacterium, its outer membrane give protection to them from antibacterial drugs. Even though the 2 trial groups were showed Zone of inhibition against both pathogens, Mean Zone of inhibition of the 2 trial groups were lower as compared to that of Mean zone of inhibition of the standard against *E. coli* and *Staphylococcus aureus*.

Student't' test was used to interpret the data depicted in Tables 5-10. Since't' value is not statistically significant at the level of $P < 0.05$, null hypothesis is accepted and alternate hypothesis is rejected. Hence it is statistically proven that antibacterial activity of Group 1- Chakramarda patra Swarasa alone is equal to that of Group 2- Chakramarda patra Swarasa and Dadhi against *E. coli* and *Staphylococcus aureus*.

We can assume that, Dadhi has more action in vyadhi hara aspect because Dadhi does not show any statistically significant antibacterial activity. In Yogamritam reference also Dadhi is taken as an Anupana. Hence we can assume that Dadhi has more action in body, especially in Gastro intestinal system. Dadhi is a rich source of calorie, micro nutrients and macro nutrients.

Probable mode of action

Chakramarda has Krimighna action hence it is having antibacterial activity. Atisara is a Vāta pradhana tridōṣaja vyadhi. Chakramarda by its Vāta Pitta Kapha (NI.R), Madhura Rasa (B.P), Ruksha guna, Agnideepan (So.Ni), Samgrahi (M.D, Ni.R) properties effectively do the samprapti vighatana of Atisara. Hence Chakramarda can be effectively used in Atisara. Quercetin and Emodin present in the *Cassia tora* showed proved antibacterial activity. Dadhi is having Amla rasa, Guru, snigdha guna, Amla vipakam, Samgrahi, Deepana, Balavardhana, Aruchi nashana, Vātamayaghna. Godadhi is used in this study it is Snigdha, Madhura vipakam, Deepana, Balavardhana, Vātapaham, Pavitram, Ruchipradam. It is a rich source of energy, Micro and Macro nutrients.

CONCLUSION

The present study was aimed to evaluate the antibacterial activity of Chakramarda patra Swarasa alone and Chakramarda patra Swarasa along with Dadhi in selected Diarrheal causing pathogens such as *E. coli* and *Staphylococcus aureus*.

The following conclusions can be drawn from the study:

The first group Chakramarda patra Swarasa alone and the second group Chakramarda patra Swarasa along with curd were found to be effective against both *E. coli* and *Staphylococcus aureus*. But Mean Zone of inhibition of the 2 trial groups was found to be lower as compared to that of Mean zone of inhibition of the standard against *E. coli* and *Staphylococcus aureus.*

Future scope of the study

In vivo and clinical studies can be carried out to find out the effect of this simple combination against the diseases caused by these 2 pathogens *E. coli* and *Staphylococcus aureus* in Diarrhoea as well as the diseases other than Diarrhoeal infection and to find out the mechanism of antibacterial activity of this combination.

Since this combination was found to be effective against gram negative *E. coli* bacteria, further studies can be done to find out its action against other gram negative bacteria.

REFERENCES

1. Sushruta, Sushruta Samhita (Nibandha Samgraha commentary); Dalhana, Varanasi, Chaukhamba Surbharati Prakashan; Reprint 2010, Uttara tantra-40th chapter, Atisara Pratishedha; 2010. p. 616-710.
2. D. Sreeman Nampoothiri, Yogamritam, Alappuzha, Vidhyarambham Publishers; 2006. p. 116.
3. P.C Sharma, M.B Yelne, T.J Dennis. Data Base On Medicinal Plants used in Ayurveda, Vol, New Delhi, Central council of Research in Ayurveda and Siddha, edition; 2001. p. 111-118.
4. FSSAI Manual of Methods of Analysis of Foods Milk and milk products, Food safety and standards authority of India, New Delhi, Ministry of health and Family
5. K. Narayana Aiyar, A.N. Namboodiri, M. Kolammal. Pharmacognosy of Ayurvedic Drugs (Kerala), Trivandrum, The Central Research Institute, University of Travancore 1957; Vol 8: 70, 71.
6. Al-Shawany AW. Alcoholic and phenolic compound activity of *Piper nigrum* against some human pathogenic bacteria. Biomedicine and biotechnology 2014; 2(1): 20-28.

7. Malati G Chauhan, A; P.G Pillai, Microscopic Profile of Powdered Drug Used In Indian System of Medicine, Jamnagar, Institute of PG teaching and research in Ayurveda, Gujarat Ayurveda University; 2007. p. 34.

Cite this article as:

Sreelekshmi S *et al*. Efficacy of Chakramarda patra Swarasa (*Cassia tora* Linn) and Dadhi in Krimija atisaram: A Preclinical Approach. Int. J. Res. Ayurveda Pharm. 2020;11(5):212-217 http://dx.doi.org/10.7897/2277-4343.1105171

Source of support: Nil, Conflict of interest: None Declared