AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems
Research Article

A CRITICAL STUDY ON *KARYA- KARAN VADA* IN RELATION TO GRAM NEGATIVE MICRO-ORGANISM IN UTI

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KEYWORDS: *Aghimantha*, Gram Negative Microorganism, *Karya Karana Vada Padma*, *Priyangu*.

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ABSTRACT

The doctrine of cause & effect (*Karya-Karana Vada*) is well versed and common in ancient Indian Philosophy *Sankhya Darsana* which is clinically adopted in *Ayurveda*. *Karya-Karana Sidhanta* is applicable for both *Swastha* and *Vikara avastha*. Therefore the study was conducted to evaluate the critical doctrine of Cause and Effect in the light of its clinical entity, and the efficacy of *Padma* (*Nelumbium speciousum* Willd), *Priyangu* (*Callicarpa macrophylla* Vahi) and *Agnimantha* (*Premna intergrifolia* Linn) on gram negative organisms to establish the stipulated doctrine i.e. *Karya-Karana Vada*. This was a in-vitro study conducted on the microorganisms causing UTI commonly E. coli sp. and Klebsiella sp. The Bacteria Escherichi coli species and Klebsiella species were collected separately from the stock culture of Pathology Laboratory. Crude and Sterile Plant extract of above plant are used for the study. The crude and the sterile extract of the selected plants named *Padma, Priyangu* and *Agnimantha* are effective to inhibit the zone of colonisation of E.coli & Klebsiella in varying degree. These studies were performed in triplicate. This study concluded that micro organism enter into the bladder and kidney through urethra or any others means, they multiply in urine and change urine pH, causes Urinary Tract Infection. The responsible above said two gram negative micro-organism may or may not produce UTI, it depends upon the potentiality causative factors as justified in the doctrine of Cause and Effect (*Karya-Karana Vada*), and above mention three herbs shows significant anti microbial activities.

INTRODUCTION

The doctrine of cause & effect (*Karya-Karana Vada*) is well versed and common in ancient Indian Philosophy *Sankhya Darsana* which is clinically adopted in *Ayurveda*. The *Karana* (cause) is that which is invariably and unconditionally antecedent to the *Karya* (action). The primary aim of this science is *Dhatu samya kriya*[1] where as the means of doing the same is *Samanyadi* six elements i.e., *Samanya*, *Visesa*, *Guna*, *Karma*, *Dravya*, and *Samavaya*[2]. So in *Karya Karana* relationship *Rogarupa Karya* and *Dhatu samya* rupa *Karya* are consider. *Dhatu vaisamya* is cause due to *Visama hetu* where as *Samadhatu* is responsible for *Dhatusamya*[3]. *Karya –Karana Sidhanta* is reveled to know both *Swastha* and *Vikara hetu* and likewise the *lingas* or manifesting feature of *Dhatu samya* and *Dhatu vaisamya* are *Karya*.

The *Vata*, *Pitta* and *Kapha* alone, either separately or conjugally produces endogenous diseases though exogenous disease may also be accompanied by the vitiation of *Vata*, *Pitta* and *Kapha*[4]. Ultimately the disturbances of the homeostatic condition take place for the causation of the disease. The exogenous diseases at a certain stage disturb the equilibrium of *Dhatus* and this disturbance in the equilibrium is also a subsidiary factor to designate as an intermediary causative factors[5]. *Ayurveda* classics have not been mentioned the term microbes or microbial activities has not been clearly described in the text but similar concept of microbes and their functional activities and remedies have been vividly described in term of *Krimi*.[6]

Mutra is termed as *Drava mala* (liquid waste product) and if there is any abnormality in its formation and excretion, then they may lead to the production of the diseases like *Mutrakrichha* and *Mutraghat*. These diseases due to *Mutravaha srotodusti* used to get immense importance in *Samhita* period[7]. *Mutravaha srota* in terms of urethra, bladder, ureter, kidney or as a whole urinary tract[8] gets deranged due to some micro-

organism and are terms as Urinary Tract Infection (UTI), manifested with frequent feeling to urinate, pain & burning sensation during micturation and cloudy urine[9].

The main and common agents for the production of UTI are Escherichia Coli species and Klebsiella spacies[10]. All though urine contains a variety of fluids, salts and waste products, it does not usually have bacteria in it. When bacteria get into the bladder and kidney through urethra or any others route, the micro-organism multiply in urine causes Urinary Tract Infection[11]. The responsible above said two gram negative micro-organism may or may not produce the aforesaid infections; it depends upon the potentiality of the factors as justified in the doctrine of Cause and Effect (*Karya-Karana Vada*). Though a few numbers of works has already been carried out in this connection of with effects of drugs having antimicrobial property in Urinary Tract Infection but no such work following the doctrine of *Karya -Karana Vada* and effect of the selected three drugs on UTI has been carried out in vitro study model.

The respective microorganisms causing UTI in the community include E. coli derived from the gastrointestinal tract (about 75% of infections), Proteus spp. Pseudomonus Spp. Streptococcui and Stphylococcocus epidermidis. In hospital, E coli still predominates, but Klebsiella or Steptococcoci are more common. UTI are very common for the prevalence of the disease and subjected to the host response.[12]

Intake of foods, drinks and sexual intercourse when having the urge for micturation especially by those suffering from wasting and consumption[13]. Causes of *Mutrakriccha* (dysuria) are exercise (in excess of one's own capacity), intake of medicaments having *Tikshna* (Sharp) attributes & *Rukhya* (un-unctuous) ingredients in excess; habitual intake of alcohol, intake of the meat of animals inhabiting marshy land (*Anupa mamasa*) and fish in excess, Regular riding over back of the fast moving animals, Intake of food before the previous meal digested; indigestion (Chronic).[14]

Urine flow and normal micturation wash out bacteria. Urine stasis in terms of *Mutra* & *Shukra vega dharan*, promotes UTI. The characteristic features of *Mutra*[15] and *Shukra*[16] *vega dharana* are pain in the bladder and phallus, dysuria, headache, bending of the body and distension of lower abdomen and the pain in the phallus and testicles, malaise, cardiac pain and retention of urine are caused by the suppression of the seminal discharge respectively.

Therefore the study will be carried out with the following aims and objects.

a. To evaluate the critical doctrine of Cause and Effect in the light of its clinical entity.
b. To evaluate the efficacy of *Padma* (*Nelumbium speciousum* Willd), *Priyangu* (*Callicarpa macrophylla* Vahi) and *Agnimantha* (*Premna intergrifolia* Linn) on gram negative organisms to establish the stipulated doctrine i.e., *Karya-Karana Vada*.

METERIAL AND METHODS

The experimental study was conducted in the laboratory of the institute of post graduate Ayurvedic Education and Research at SVSP Hospital, 294/3/1, APC Road. Kolkata- 09, Department of Sharir Samhita. The plants were selected for this study based on their medicinal uses- *Padma* Leaves, *Priyangu* Flowers, *Agnimantha* Root Barks. The plant were collected and properly identified by the Apothecary department of IPGAE&R AT SVSP Hospital.

Preparation of Crude and Sterile Plant Extract

At first leaves of *Padma* were measured and then thoroughly washed in sterilized water. Then leaves were placed on mortar and crushed into juice with the help of pestle. After crushing for 10 – 15 minutes fresh juice was extracted and collected in sterilized test tube. The collected material was 10ml, measured through measuring cylinder. From the 10ml of crude extract of *Padma*, 5ml was filtered through sterile seitz filter, placed on sterile conical flask of 50ml for sterilization without heating. The complete sterilization had done after 3 hrs. Now both types of extract (crude and sterile) of *Padma* were ready to prepare for making diffusion disc.

(A) Making of Diffusion Disc: For making of Diffusion Disc, Whatman No. 1 filter paper was bought from reputed medical equipment shop and punched out into 6mm disc by 6mm punching machine. In this technique 1000 disc were made and autoclaved for 15 minutes. Now 0.5ml of freshly extracted crude and sterile juice of *Padma* were placed in two separate 1'' sterile petridish by 0.5ml micro pipette which had been already sterilized.

After 15 minutes autoclaving the disc were ready to use. 50 numbers of sterile discs were placed evenly in each 1'' sterile petridish containing 0.5ml both crude and sterile extracts of *Padma* by forceps which had been already sterilized by heating through sprit lamp.

The both the petridish containing disc were stored in cool and dry place in normal temperature. By this process discs, impregnated with crude and sterile extracted of a *Padma* were allowed to dry. After 36 hrs the discs become ready for susceptibility testing. In the above process 50 numbers of diffusion disc impregnated with both crude and sterile extract of *Padma* separator were made. Likewise extract of *Priyangu* (*Callicarpa macrophylla* Vahl) and *Agnimantha* (*Premna integrifolia* Linn) were made and their respective disc in both pattern were prepared.

(B) Collection and Culture of Bacteria: The Bacteria Escherichi coli species and Klebsiella species were collected separately from the stock culture of Pathology Laboratory, Department of Pathology, Institute of Post Graduate Ayurvedic Education & Research, at S.V.S.P. Hospital, Kolkata 700009; they were examined Bio chemically and morphologically.

At first 4 – 5 well isolated colonies of E. coli were selected from the stock culture of E. coli having same morphology type. Then at a temperature was made to touch the top of each colony with a microme wire loop,

sterilized by heating through spirit lamp. Microme loop containing E. coli growth was transferred to a sterile tube containing 1ml of nutrient broth medium and stirred properly for a few seconds. The tube contain broth culture was allow to incubate for 2 hrs at 350C temperature in incubator until it achieved turbidity. Likewise the culture broth of Klebsiella species was also made in the above process. Now both the culture broths of Bacteria E. coli species and Klebsiella species were ready for susceptibility test.

(C) Disc Diffusion Susceptibility Testing: First 20gm of Nutrient agar was liquefied by heating through warm water bath and poured evenly in each 4'' sterile petridish divided in equal quantity. The both petridish had already been divided in three quadrants namely 'A', 'B', 'C' for *Padma, Priyangu* and *Agnimantha* respectively by marking lower external surface of each petridish. The liquid nutrient agar became condensed after 5 minutes in normal temperatures. Now 2 inculums suspensions of both E. coli species and Klebsiella species separately cultured were flooded evenly in both nos. (1) and (2) petridish respectively. After that previously prepared diffusion disc impregnated with crude and sterile extract of *Padma, Priyangu* and *Agnimantha* separately were placed in respective zone of both petridish nos. (1) and (2) by sterile forceps. Forceps were sterilized again in each time after placing one disc in respective zone by heating method with the help of sprit lamp. then three discs were placed in triangular fashion in which two centers of the discs apart from 30mm distance. The discs loaded with both type of extracts were allowed to diffuse for 5 minutes and both nos. (1) and (2) petridish were kept for incubation at 370C for 18 – 24 hrs.

At the end of the incubation, both the petridiseh were collected from the incubator. Inhibition zone formed around the discs were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Table 1: (1st Experiment) Effect of crude and sterile extract of *Padma, Priyangu* and *Agnimantha* on E. coli Sp. and Klebsiella Sp.

Plant	E. coli Sp.		Klebsiella Sp.	
	Crude	Sterile	Crude	Sterile
	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]
Padma	10.23 ± 0.65	9.67 ± 1.53	14.13 ± 0.90	14.07 ± 1.42
Priyangu	9.37 ± 2.12	14.33 ± 2.08	10/93 ± 1.4	1.83±1.55
Agnimantha	9 ± 1	11 ± 1	10.03 ± 1.05	10.67 ± 1.05

P<0.05, S.D-Stranded deviation of mean

Table 2: (2nd Experiment) Effect of crude and sterile extract of *Padma, Priyangu* and *Agnimantha* on E. coli Sp. and Klebsiella Sp.

Plant	E. coli Sp.		Klebsiella Sp.	
	Crude	Sterile	Crude	Sterile
	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]	(Mean±S.D.) [mm]
Padma	10.23 ± 0.68	12.43 ± 0.51	14.47 ± 1.59	14.33 ± 1.60
Priyangu	12.87 ± 1.02	14.83 ± 0.72	11 ± 1.25	10.33 ± 1.57
Agnimantha	9.06 ± 0.90	8.9 ± 1.49	9.47 ± 0.70	8.67 ± 2.08

P<0.05, S.D-Stranded deviation of mean

Table 3:(3rd Experiment) Effect of crude and sterile extract of *Padma, Priyangu* and *Agnimantha* on E. coli Sp. and Klebsiella Sp.

Plant	E. coli Sp.		Klebsiella Sp.	
	Crude	Sterile	Crude	Sterile
	(Mean ± S.D.) (mm)	(Mean ± S.D.) (mm)	(Mean ± S.D.) (mm)	(Mean ± S.D.) (mm)
Padma	11.4 ± 0.72	12.43 ± 0.51	15.1 ± 0.95	16.1 ± 1.15
Priyangu	13.9 ± 1.05	15.2 ± 0.80	11.33 ± 1.15	10.73 ± 0.30
Agnimantha	10.13 ± 0.53	8.63 ± 1.25	9.67 ± 0.76	9.77 ± 1.12

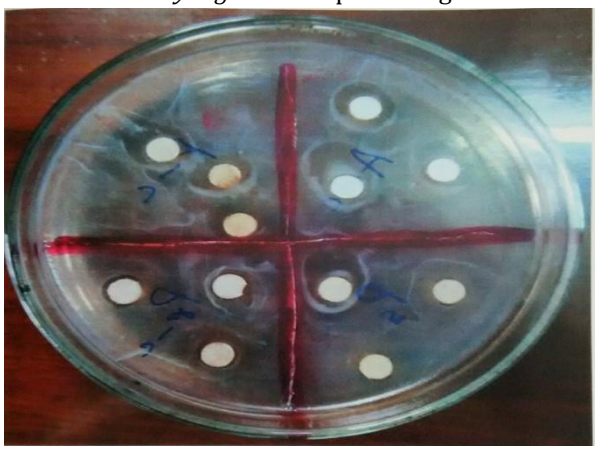
P<0.05, S.D-Stranded deviation of mean

Observation and Discussion: It is observed that

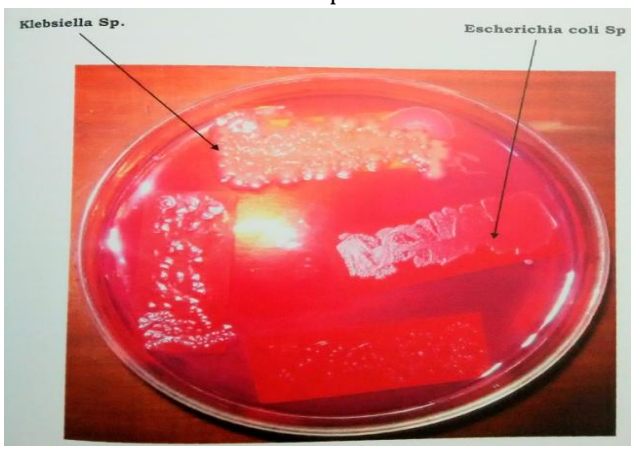
1. The sterile extract of *Priyangu* is more effective to inhibit the colonisation of E. coli in comparison to the effect of *Padma* and *Agnimantha.*
2. That the sterile extract of *Padma* is more effective to inhibit the colonisation of Klebsiella in comparison to the effect of *Priyangu* and *Agnimantha*.
3. That the crude extract of *Priyangu* is more effective to inhibit the colonisation of E. coli in comparison to the effect of *Padma* and *Agnimantha*.
4. That the crude extract of *Padma* is more effective to inhibit the colonisation of Klebsiella in Comparison to the effect of *Priyangu* and *Agnimantha*.

On the basis of the above observation it may be revealed that the crude and the sterile extract of the selected plants named *Padma, Priyangu* and *Agnimantha* are effective to inhibit the zone of colonisation of the

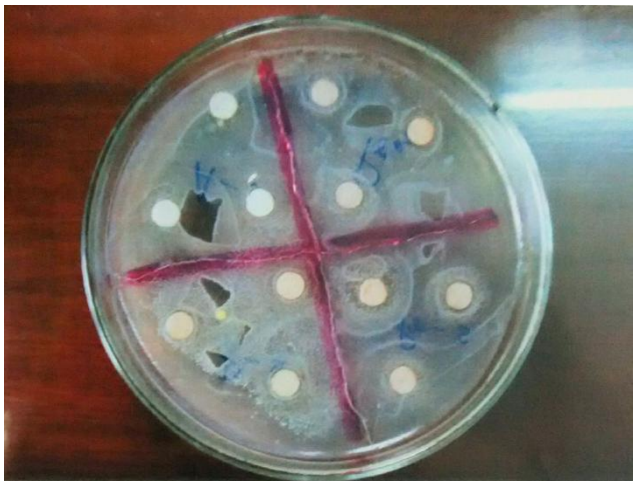
microorganism (E.coli & Klebsiella) in varying degree. The effectiveness of the crude and sterile effect of *Priyangu* to inhibit the colonisation of E. coli and the crude and sterile effect of *Padma* to inhibit the zone of colonisation of Klebsiella are the suggestive of antimicrobial activity. The highest effectiveness of the sterile extract of *Priyangu* in compromising to effect of other two drugs either crude or sterile from proves the qualities of the Ayurvedic formulation in context to Antimicrobial activity is accelerated may be due to that during the sterilization process of those drugs, through seitz's filter some effective constituent are being radically activated which are not profound in context to the administration of the plants in its crude form.



Zone of inhibition of Escherichia Coli Sp



Growth Culture of Klebsiella Sp & Escherichia Coli Sp



Zone of inhibition of Klebsiella Sp.

CONCLUSION

The *Karana* (cause) is that which is invariably and unconditionally antecedent to the *Karya* (effect). The disease is produced due to the intrinsic and extrinsic factors which are endogenous and exogenous in nature respectively. The profoundness of the disease process is magnified through the intense causative factors. The produced disease is manifested with the different characteristics because of its varying pathogenesis and its quality of genesis. The generated disease is amplified in respect to the resistance of the body mechanism, therefore the causative factors of the relevant diseases are classified in different ways. The significance of *Nidana* plays the fundamental role for the production and diagnosis of the disease as a separate entity and outcome result of the five respectively.

The main and common agents for the production of UTI are Escherichia coli species and Klebsiella species. All though urine contains a variety of fluids, salts and waste products, it does not usually have bacteria in it. When bacteria get into the bladder and kidney through urethra or any others route, the micro-organism multiply in urine and causes Urinary Tract Infection. The responsible above said two gram negative micro-organism may or may not produce the aforesaid infections; it depends upon the potentiality of the above said and other respective factors as justified in the doctrine of Cause and Effect (*Karya-Karana Vada*). *Vikara vighat abhava, Mutravaha srota dusti* and Bacterial Adhesion, *Rakta dusti, Mutra dusti, Bala Kshay, Viruddha ahara* and *Oushad sevana, Mutra & Shukra vega dharana* etc are the respective causative factors for the production of UTI.

The *Chala guna* of *Apana vayu* initiate to excretion of urine. Due to impaired function of *Jathargni*, either the *Mutra* is not formed properly or due to the impaired function of *Apana vayu*, the formed *Mutra* is not properly excreted out. The impaired function of *Bhutagni* and *Dhatwagni* also causes the Malabsorption and depletes metabolism, resulting the formation of *Ama* at the site of *Mutravaha srota* which is pre susceptible to

the disease *Mutrakriccha*. Disruption of this uro-epithelium by trauma predisposes to UTI. The trauma in the urogenital tract is compared *Raktaja mutrakriccha*, because of the excessive vitiation of the blood, it may be due to the invasion of the micro organism after traumatic injury and may be as a result of excessive sexual intercourse. Both *Priyangu* and *Padma* are having anti-microbial activity in respect to E. coli and Klebsiella respectively.

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Cite this article as:
De Swarup, Khatun Hazera, Chattopadhyay Abichal, Mukherjee Gopeswar. A Critical Study on Karya-Karan Vada In Reletion to Gram Negative Micro-Organism in UTI. AYUSHDHARA, 2016;3(5):857-861.
Source of support: Nil, Conflict of interest: None Declared