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PRECLINICAL STUDY ON URINARY TRACT INFECTED EXPERIMENTAL RATS TREATED *SPHAERANTHUS INDICUS L.*

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ABSTRACT: A model of ascending Urinary tract infection (UTI) in rats was developed to study the significance of antibacterial activity of *Sphaeranthus indicus* L. and compared with standard antibiotic ofloxacin as a positive control. This plant used in folk medicine as a remedy for urinary tract infections is belong to Asteraceae family. Outbred female wistar rats were used throughout the study and *Escherichia coli* was used as pathogen isolated from the urine of patients suffering from urinary tract infections. Methanolic extract (300 mg/mL) and aqueous extract (300 mg/mL) of *Sphaeranthus indicus* were administered orally for 15 days. After 15 days of treatment of the infected rats with herb extract, bacterial count in the urine, immunoglobulin G and M levels were estimated, and his pathological changes of kidney, liver, and urinary tract were observed. In the disease control group bacterial infection has increased than that of the normal control group. In the treated group with aqueous extract (300 mg/mL) & methanolic extract (300 mg/mL) bacterial count has decreased considerably. There was clear dose-response of two extracts. In the study, the disease control group's immunity level has decreased than the normal control group. The rats treated with aqueous and methanolic extracts (300 mg/mL) of *Sphaeranthus indicus* has shown significant difference when compared with a disease control group. In histopathological observation, the disease control group showed considerable damage in the kidney and urinary bladder histology. This observation is an indication of very effective kidney and urinary bladder protection against bacterial infection by *Sphaeranthus indicus*

INTRODUCTION: Urinary tract infection (UTI) is a condition when any portion of the urinary tract (urethra, bladder, ureter, and kidney) gets contaminated with microbes or every so often with fungus [1].

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It is the most common disease after respiratory tract diseases. There are a few approaches utilized to treat and avoid persistent and repetitive urinary tract infections, *i.e.*, taking antibiotics, bioactive common nourishments, utilizing probiotics, and keeping up great individual hygiene [2].

For the animal model study, mice are chosen for these disease models since they appear to be touchy to numerous of the same bacterial properties which cause UTI in humans [3]. The small animal model study of urinary tract infection compares the host immune response in female and male animals by

transurethral catheterization [4]. Herbal remedies are very helpful in treating Urinary tract infections, in relieving discomfort, and encouraging quick recovery. These remedies may help patients get through an existing or recurrent urinary tract infection without the use of antibiotics. Since urinary tract infection is a result of the immune system weakness, which does not defend against foreign bacteria that enter the urinary tract and causes an infection to develop in the urinary tract, bladder & kidney. Herbal remedies are assumed to enhance resistance to infection by stimulating the immune system so that the illness can be resolved as rapidly and with as little discomfort as possible. The immune system is known to be involved in the etiology as well as pathophysiological mechanisms of many diseases [5, 6].

Herbs have many different actions that can help in the treatment of urinary tract infection. Clinical and epidemiological studies support the use of cranberry in maintaining a healthy urinary tract [7]. In vivo and *in-vitro* antibacterial activities of cranberry extract observe against *E-Coli* in Urinary tract Infected rat [8]. Vaccinium myrtillus (Bilberry; Blueberry) extracts possess anti-adhesive effects against uropathogenic bacteria.

Arctostaphylos uva ursi (Blueberry) fruit commonly used for its antimicrobial property in controlling UTIs [9]. Several other herbs that are used for the treatment of UTIs but lack of scientific basis include *Agrimonia eupatoria* (agrimony), *Althea officinali*s (marshmallow), *Apium graveolens* (celery seed), *Arctium lappa* (burdock), *Elymus repens* (couchgrass), *Hydrangea aborescens* (hydrangea), *Juniperus communis* (juniper), *Mentha piperita* (peppermint), *Taraxacum officinalis* leaf (dandelion), *Ulmus fulva* (slippery elm) and *Zea mays* (corn silk) [10].

Sphaeranthus indicus L. is a medicinal plant widely used in the Indian traditional system of medicine for curing various ailments. This plant is used in folk medicine as a remedy for urinary tract infections [11]. Phytochemical and bio autographical studies revealed the presence of medicinally important constituents like essential oil and terpene proved as antibacterial components [12, 13]. The objective of the new sesquiterpene glycoside and sphaeranthanolide were isolated from the flowers of *Sphaeranthus indicus*, and it was found to be an immune stimulant [14]. Diuretic activity of *Sphaeranthus indicus* Linn. ethanol extract was observed in albino rats [15]. In the present study, a model of urinary tract infection (UTI) was developed in a rat to evaluate the antibacterial effect and immunomodulatory effects of the plant *Sphaeranthus indicus* L.

MATERIAL METHODS:

Plant Collection: Fresh plants and plant parts were collected randomly from the field area of Surat and Valsad, Gujarat, India. Taxonomist and Voucher specimens confirmed the taxonomic identities of these plants are deposited in the department herbaria (Voucher specimens no: BVBRC-171). Fresh plant materials were washed under running tap water, air-dried and homogenized to a fine powder, and stored in an airtight bottle.

Preparation of Crude Plant Extract: Solvent extracts were prepared by adding 250 g of plant powder to 500 mL of solvent in the conical flask then kept on a rotary shaker for 24 h for proper extraction. The solutions were then heated at 45 °C on a water bath for about 10 min. The content was then filtered with Whatman No.1 filter paper, and the filtrate was sterilized using autoclave and concentrated in a water bath at 45 °C for about 10 min. The filtrate was concentrated under reduced pressure at 80 °C using the rotary evaporator to make a final volume of one-fourth of the initial volume and finally dried at 55 °C in the oven for three days. Dried extracts were weighed and stored at 4 °C in the refrigerator for future use [16, 17].

Animal Welfare: (CPCSEA) guidelines for Laboratory Animal Facility use animals to conduct study on plant effect has been reviewed and approved by the Institutional Animal Ethics Committee (Protocol No. FLAIR/IAEC/002/006/2012).

Animals: Outbred female wistar rats (weight 150 ± 20 g) were used throughout the study. They were kept under standard environmental conditions. Four animals were housed in each cage and fed with a standard diet and water. The animals were allowed to acclimatize for a period of a minimum of 24 h prior to introducing the inoculums.

Animal Room Conditions: Experimental animals were housed in an animal facility with adequate

environmental conditions of temperature 22 + 3 °C and relative humidity 30-70%. The 12 h light and 12 h dark cycle were maintained manually throughout the study.

Acclimation: Animals were acclimatized for a minimum period of seven days and observed for general health before the commencement of the experiment.

Bacteria: The *E. coli* strains to be used in the study were isolated from patients with UTIs.

Bacterial Growth Conditions: Bacteria from frozen stock were grown at 370 °C for 48 h. The bacterial culture was transferred to the fresh Nutrient broth and incubated overnight at 370 °C. The bacteria was incubated at 37 °C, shaken at 200 rounds/min overnight, and centrifuged at 6,500 × g for 10 min. The pellet will then suspended in phosphate-buffered saline (PBS) to a concentration of approximately 108 CFU/ml [18].

Preparation of Rat Model For Inducing UTI: For the inoculation, seven groups were used in which 24 rats were inoculated transurethral with the help of a catheter. The catheters were made from tubing (autoclavable; inner diameter, 0.30 mm; outer diameter, 0.64 mm), the bladder of each animal was emptied by gentle pressure on the abdomen before inoculation. The appropriate bacterial suspension was then instilled into the bladder while animals were kept under ketamine anaesthesia. For bladder inoculation, a 1.25-cm soft polyethylene syringe fitted with The pieces of 1.2 cm catheter tubing was placed on hypodermic needles (26 gauge) The tip of the needles were cut had to be perfectly smooth so that it did not cause tissue injury during inoculation and the catheter was carefully pushed in horizontally until it reached the top of the bladder, and 0.05 ml of bacterial suspension was introduced into the bladder. The catheter was removed immediately after inoculation [18, 19].

Colony-Forming Unit (CFU) Assay: For bacterial count determinations. After three days of inoculation, urine from each rat was collected in Eppendorf tubes by gentle compression of the abdomen, and 1 µl was inoculated on agar plate for counting the bacterial colony.

Treatment and Experiment Design: Group-A which served as the normal control group, was administered orally 0.25% Na- CMC (sodium salt of carboxymethyl cellulose) per kg body weight and group-B UTI infected group served as disease control (for counting bacterial load) no treatment was given to them, group-C was used as standard antibiotic group ofloxacin (0.32 mg/kg) was administered orally and Group-D was administered water extract 300 mg/kg body weight. Group-E to G was respectively administered orally with 100, 200, 300 mg/kg body weight of extract of *Spheranthus indicus*, by subcutaneous (dissolved in 0.25% CMC-Na) for 15 days. The volume of a dosage administered were 5mL/kg body weight.

TABLE 1: ANIMAL EXPERIMENTAL GROUP DESIGN

S. no.	Group	Treatment	Doses conc.	Dose volume(mL/kg)	No of Animals	No of Days
1	A	0.25% CMC-Na	--	5	04	15
2	B	Bacterial Suspension	10^8 CFU/ml	0.05ml	04	3
3	C	Ofloxacin	0.32 mg/Kg	5	04	5
4	D	Water extract of SI	300 mg/Kg	5	04	15
5	E	SI Treated	100 mg/Kg	5	04	15
6	F	SI Treated	200 mg/Kg	5	04	15
7	G	SI Treated	300 mg/Kg .	5	04	15

(SI -*Spheranthus indicus*,)

Bacteriological Examination: After five & fifteen days of plant extract, administration assessment of bacterial inhibition in treated animals were carried out. Urine will be collected by gentle compression of the bladder through the abdominal wall.

A sample of urine were be collected from each rat was collected in eppendorf tubes by gentle compression of the abdomen and 1 µl was inoculated on agar plate for counting the bacterial colony. The plates will be incubated overnight at 37 °C and colonies will be counted for the bacterial load [18, 19].

Assessment of Immunological Effects After 15 days of treatment with extract hematological

parameters, Immunoglobulin G (IgG) and Immunoglobulin M (IgM) were evaluated; blood samples were collected from each animal by retina puncture into plain sterile tubes blood samples were centrifugation at 2000 rpm for 15 min and serum was used for testing of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) by the QUANTIA-IgG & the QUANTIA-IgM kit method.

Statistical Analysis: Data were expressed as mean ± S.D. The statistical significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) followed by a post hoc Tukey HSD test. A probability level of 0.05 or less was accepted as significant.

Histopathological Studies: After fifteen days of treatment, rats were sacrificed by cervical dislocation. The liver, kidney, and bladder were carefully dissected from the abdominal region. They were fixed in 10% formaldehyde and sliced into a thickness of 2.1 mm. The tissues were dehydrated with alcohol of graded concentrations. They were further treated with paraffin wax, and sections of the tissues were then cut on a microtome to 5 µm. These were later attached to a slide and allowed to dry. The sample slides were subsequently stained in hematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded [18].

RESULT AND DISCUSSION:

Bacteriological Examination: After 5 and 15 days of treatment of the infected rats *with Spheranthus indicus* herb extract, the bacterial count in the urine was estimated. In the disease control group, the bacterial infection has increased than that of a normal control group. In the treated group with aqueous extract (300 mg/mL) & methanolic extract (300 mg/mL) bacterial count has decreased considerably.

There was a clear dose-response of all the extracts. After five days of treatment with *Spheranthus indicus* methanolic extract groups with 100, 200 and 300 mg/ml, the bacterial count was 38.13%, 66.63%, and 90%, respectively. After fifteen days of treatment similar decreasing trend was observed in all three concentrations. Even the aqueous extract at a dose of 300 mg/ml has shown a similar effect as a methanolic extract by reducing the bacterial count by more than 90% **Fig. 1.**

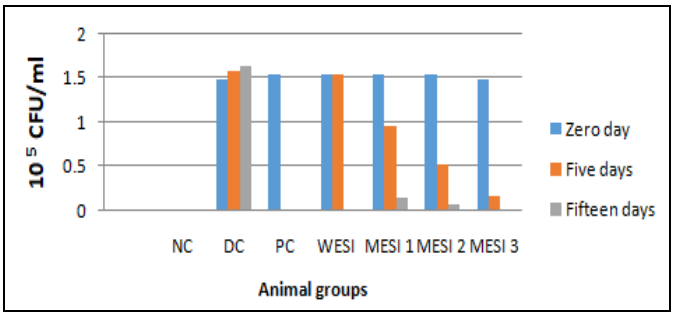

FIG. 1: BACTERIAL COUNT AFTER TREATMENT WITH *SPHERANTHUS INDICUS* EXTRACT OF UTI INFECTED

NC Normal Control DC: Disease control, PC: Positive Control WESI: Water extract of *Spheranthus indicus* 300 mg/mL, MESI1-Methanolic extract of *Spheranthus indicus* 100 mg/ml, MESI2-Methanolic extract of *Spheranthus indicus* 200 mg/mL, MESI3-Methanolic extract of *Spheranthus indicus* 300 mg/ml)

Evaluation of Immunomodulatory Activity: After 15 days of treatment of the infected rats with *Spheranthus indicus* herb extracts, immunoglobulin G and M levels were estimated. In the disease control group, the immunity level has decreased than the normal control group. The rats treated with aqueous and methanolic extracts 300 mg/ml of *Spheranthus indicus* have shown a significant difference compared with a disease control group similar to a normal control group. Methanolic extracts 100 mg/ml & 200 mg/ml of *Spheranthus indicus* have shown a significant difference compared with a disease control group. All the treated groups showed clear dose-response. In antibiotic-treated groups, Immuno-globulin M (IgM) and Immunoglobulin G (IgG) levels have increased.

Histopathological Studies: The liver, kidneys, and urinary bladder were removed from the rats' bodies at the end of the treatment. The section of each organ was observed for Histopathological study. His pathological changes of kidney and bladder were observed, all groups of the liver showed normal structure. Photomicrograph of the disease control groups both kidneys shows degenerative cellular changes, focal inflammatory cells infiltration. A few glomerulus show dilatation. Focal tubular necrosis and a few tubules show necrotic debris in the lumen, increased vascularity in the stroma. Overall histology was suggestive of

acute or chronic glomerulonephritis, the group of animals treated with antibiotic & aqueous extract of *Spheranthus indicus* for fifteen days no such pathological damage were observed, the group treated with methanolic extract of *Spheranthus indicus*, have shown in both the kidney mild asymmetrical dilation glomeruli and slightly increased vascularity and almost normal looking renal tubules which were indicated some recovery shown in the treated group.

Photomicrograph of the disease control groups the urinary bladder showed a bladder wall was slightly thickened, focal mononuclear and focal infiltration of inflammatory cell in submucosal area. The groups of animals treated with antibiotic & aqueous extract of *Spheranthus indicus* for fifteen days no such pathological damage was observed in the bladder. The groups treated with methanolic extract of *Spheranthus indicus* have shown mild focal infiltration of inflammatory cell in sub mucosal area, indicating some recovery shown the treated group. The pathophysiological changes of the kidney and bladder also confirm that our model is true for UTI infection. The model we have adopted is a simple and reliable method of inducing UTI in rats. It is observed that the plant extracts have shown a drastic decrease in urine bacterial count with 5 days of treatment, which was comparable with the positive control using antibiotic ofloxacin.

A clear dose-response was also observed, which supports the pharmacological actions of the herbal extract. Another mechanism of reducing the bacterial infection urinary tract is washing of with excessive urine production. The plants studied Spheranthus indicus is reported to have diuretic and anti-inflammatory activity, and hence these activities might be one of the mechanisms in reducing the urinary tract infection,

TABLE 2: EFFECT OF SPHAERANTHUS INDICUS ON IGG AND IGM LEVEL IN UTIS INFECTED RATS

S. no.	Group of animals	IGG mg/lit	IGM mg/lit
		Mean ± SD	Mean ± SD
1	Normal control	4.3508±0.606	0.9133±0.071
2	Disease control	2.3710±0.351	0.2495±0.112
3	Positive control	4.7315±0.188*#	1.65±0.258*#
4	WESI 300 mg/mL	4.8218±0.12*#	1.61±0.374*#
5	MESI 100 mg/mL	4.2340±0.220#	1.25±0.151#
6	MESI 200 mg/mL	4.6078±0.277#	1.39±0.045#

(MESI-Methnolic extract of *Spheranthus indicus*, WESI- Water extract of *Spheranthus indicus*, Values are presented as mean ± SD of four animals. $P < 0.05$ consider to be significant difference when* Compare with Normal control group & treated group and # Compare with disease control group and treated group)

CONCLUSION: The present study of ascending unobstructed UTI with *E. coli* employed without mechanical manipulation and with little trauma or injury to the urinary tract was established as a very good model where there is a bacterial infection in the bladder and up to the kidney. *Spheranthus indicus* is proved to be very effective against urinary Tract infection.

The mechanisms must be by their potentiality as immunomodulators [20]. Their diuretic activity and may even the anti-adhesive properties might be contributing to some extent. Hence, these plants are conclusively proved to very effective in controlling UTI. The ethnic claim of *Spheranthus indicus* being used against UTI is proved to correct and there is a possibility to develop a disposable drug from this plant that can be very effective in controlling UTI.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest

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