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ACUTE TOXICOLOGICAL AND ANTI BACTERIAL STUDY OF *OSBECKIA MURALIS* NAUDIN

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ABSTRACT

Osbeckia muralis Naudin is an endemic herb in Wester Ghats. It is hispid herb from Melastomaceae family. Scientific exploration of the drug has been done by acute oral toxicity study and Anti-bacterial Study. Acute oral toxicity study showed no signs of toxicity or behavioral changes. Antibacterial study with Disk diffusion method showed the action of Osbeckia muralis Kashaya in Klebseilla pneumoniae was positive.

Keywords *– Osbeckia muralis Naudin, Toxicity study, Anti-bacterial Study*

1. INTRODUCTION

Osbeckia muralis is an endemic herb in Western Ghats[1]. The drug is identified as a Melastomaceae family member[2]. Folklore practitioners from the Karopady Village, Dakshina Kannada, Karnataka claimed its action on respiratory ailments[3]. To explore the drug scientifically all the necessary steps has to be adopted. The study aims to evaluate its toxicity and anti-bacterial effect. This information about toxicity study is useful to determine the relevance of the test for the protection of the human health and the environment, and will help in the selection of an appropriate starting dose.

A limit test can be used efficiently to identify chemicals that are likely to have low toxicity, or the limit test is primarily used in situations where the experimenter has information indicating that the test material slightly to be non-toxic. Information about the toxicity of the test material can be gained from knowledge about similar tested mixtures or products, taking in to consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed.

Aqueous extract and Kashaya of *Osbeckia muralis* Naudin were used to evaluate their antibacterial effect on two organisms viz *Staphyllococi Aureus (*Gram +ve) and *Klebsiella pneumoniae* (Gram –ve) and compared to standard drug Norfloxacin.

2. MATERIALS AND METHODS

The drug *Osbeckia muralis* was collected from moodbidri area and authenticated by Botanist has been selected for the study.Acute Oral Toxicity Study has been conducted at Alvas Ayurveda Medical College, Moodbidri (IAEC certificate No. AAMC/CPCSEA/IAEC/2015-16 AL-04).Antibacterial study has been conducted at Alvas College of Medical Laboratory Technology, Moodbidri.

2.1 Acute Oral Toxicity Study

Study has been carried out as per OECD guideline425.

Limit test at 5ml/kg body weight

The use of a dose at 5ml/kg may be considered when there is a strong likelihood that results of such a test have a direct relevance protecting human or animal health or the environment.

General Procedure

a) Sample – 5 healthy albino rats of either sex with average weight of 150-200gm will be selected randomly for the study.

b) Design of the study:

Acute toxicological studies of the plant will be done on a sample of 5 Albino rats of either sex weighing 150-200gm. Among those, 1 rat will be given 4 times the *Kashaya dose* orally. After 24hrs of observation if the rat is survived, the remaining rats are subjected for study. The dosage is fixed based on the human adult dose and is converted to animal dose on the basis of body surface area ratio (Paget and Barnes table, 1964). Rats will be observed for seven days for any acute toxicity symptoms. As per the data observed, statistics will be worked out through the appropriate statistical tests (As per OECD guidelines425 and *Clara Morpugo-et-al 1971*).

Gross behaviour was also assessed in all the rats. The procedure involves assessing the observed behavior on a subjective scale awarding- scores on 0-3-point scale as per the average intensity of the phenomena observed. After administering the limit dose i.e. 5ml/kg body weight as specified in the O E C D Guidelines 425, the observations were made after every 1 hr. up to 4 hr and then at 24 hr. interval up to 72 hr of drug administration. The rats were placed one by one in the center of three concentric circles (drawn by Chalk on a rubber sheet) of diameter, 7 cm, 9 cm and 13 cm. Assessment was done for the following parameters: CNS depression, CNS stimulation

2.2 Antibacterial Study

Aqueous extract and Kashaya of *Osbeckia muralis* Naudin were used to evaluate their antibacterial effect on two organisms viz *Staphyllococi aureus* (Gram +ve) and *Klebsiella pneumoniae* (Gram –ve) and compared to standard drug Norfloxacin. Observations were recorded.[4-9]

2.2.1 Method: Well diffusion Method

Initially, the stock cultures of bacteria like Staphyllococi aureus and Klebsiella pneumoniae were revived by inoculating in Muller Hinton broth media and grown at 37ºC for 18 hrs. The Muller-Hinton agar plates were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound and antibiotic at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

2.2.2 Method: Disk diffusion Method

Initially, the stock cultures of bacteria like *Staphyllococi aureus* and Klebsiella pneumoniae were revived by inoculating in Muller Hinton broth media and grown at 37ºC for 18 hrs. The Muller-Hinton agar plates were prepared. Each plate was inoculated with

18 h old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the paper disk was made and impregnated with standard anti-bacterial chemical and testing samples (*Osbeckia muralis* Naudin Kashaya and Aqueous extract) placed in the plate at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity Study

As the five animals survived without showing any signs of toxicity, the LD50 (Medial Lethal Dose) is greater than the limit dose (5 ml/kg) and there were no signs of toxicity or behavioural changes observed when they were observed for the next 14 days. (Fig. 1) The Table No.1 contains data related to gross behavioural changes observed during testing. Increased pellet expulsion was observed it indicates possibility of anxiety producing effect. The test drug did not produce effect on other parameters studied.

Table 1: Data related to gross behavioral changes observed during testing

Group	Dose	CNS DEPRESSION							CNS STIMULATION						
		1hr	2hr	3hr	4hr	24hr	48hr	72hr	1hr	2hr	3hr	4hr	24hr	48hr	72hr
Trial	5ml	0/5*	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Control	Tap water	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

**No. of animals exhibited toxicity signs /No. of animals dosed*

3.2 Anti-bacterial study: Well diffusion method

Aqueous extract and Kashaya of *Osbeckia muralis* in different concentrations were subjected to antibacterial study with Norfloxacin as standard drug. Gram +ve and gram –ve strains were used in which *Staphyllococi aureus* and *Klebsiella pneumoniae* from both strains were selected.

Anti-bacterial study on gram +ve organism (Staphylococci Aureus)

Aqueous extract of *Osbeckia muralis* Naudin showed no inhibitions in 500µg and 1000µg concentrations and Kashaya of *Osbeckia muralis* also showed no inhibitions in 1000µg concentration. (Table no.2)

Table 2: Well diffusion Anti-Bacterial analysis on *Staphylococci aureus*

Sample	500 µg	1000 µg	MIC µg
OM-A	0	0	NF
OM-K	0	0	NF
Norfloxacin	34	*	25

Anti-bacterial study on gram -ve organism (Klebsiella pneumoniae)

Osbeckia muralis Naudin's Aqueous extract showed no inhibition in 500 µg and 1000µg concentration and Kashaya of *Osbeckia muralis* did not show any activity. (Table No.3)

Table 3: Well Diffusion Anti-bacterial analysis on *Klebsiella pneumonia*

Sample	500 µg	1000 µg	MIC µg
OM-A	0	0	NF
OM-K	0	0	NF
Norfloxacin	38	*	25

**the inhibitions zones were too big to measure*

NF- MIC not found

Note: In above tables, NF is MIC not found in the concentrations screened

Overall conclusion from both studies (gram +ve and –ve) is that aqueous extract and Kashaya of *Osbeckia muralis* has no inhibitory activity against gram +ve (Staphylococci Aureus) and gram –ve(*Klebsiella pneumoniae*) strains at 500 µg and 1000 µg concentration. (Fig No.2)

3.3 Anti-Bacterial study: Disk diffusion method

Aqueous extract and Kashaya of *Osbeckia muralis* were subjected to antibacterial study with Norfloxacin as standard drug. Gram +ve and gram –ve strains were used in which *Staphylococci aureus* and *Klebsiella pneumoniae* from both strains were selected.

Anti-bacterial study on gram +ve organism (Staphylococci Aureus)

Aqueous extract and Kashaya of Osbeckia *muralis* Naudin showed no inhibitions (Table No.4)

***Table 4: Disk diffusion Anti-bacterial analysis Staphylococci* aureus**

Sample		MIC µg
OM-A	0	NF
OM-K	0	NF
Norfloxacin 10	30	10

Table 5: Disk diffusion Anti-bacterial analysis *Klebsiella pneumonia*

Sample		*MIC µg*
OM-A	12	1000
OM-K	24	1000
Norfloxacin 10	*32*	*10*

**the inhibitions zones were too big to measure*

NF- MIC not found

Note: In above tables, NF is MIC not found in the concentrations screened

The results are presented in the table as diameter of inhibition zones in mm

OM A- *Osbeckia muralis* Aqueous extract

OM A- *Osbeckia muralis* Kashaya

Fig. 1: Acute Oral Toxicity Study

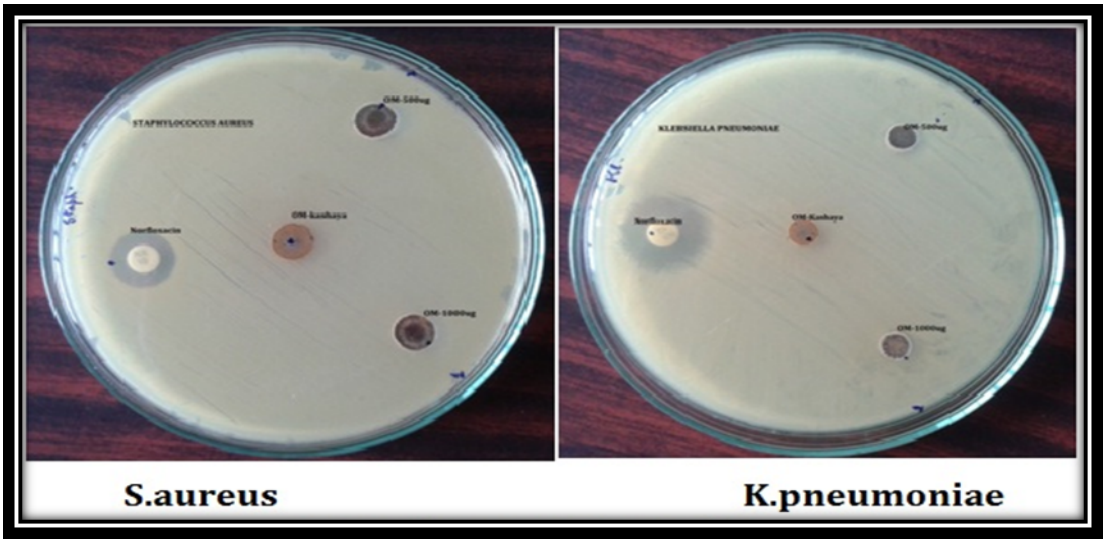

Fig. 2: Anti-Bacterial Study (Well Diffusion Method)

Anti-bacterial study on gram -ve organism (Klebsiella pneumoniae)

Osbeckia muralis Naudin's Aqueous extract and Kashaya of *Osbeckia muralis* showed inhibition in 1000µg concentration. (Table No. 5)

Overall conclusion from both studies (gram +ve and –ve) is that aqueous extract and Kashaya of *Osbeckia muralis* has no inhibitory activity against gram +ve (*Staphylococci aureus*) and in gram –ve (*Klebsiella pneumoniae*) strains both aqueous and Kashaya of *Osbeckia muralis* Naudin shows Minimum inhibition as shown in the Fig.No.3.

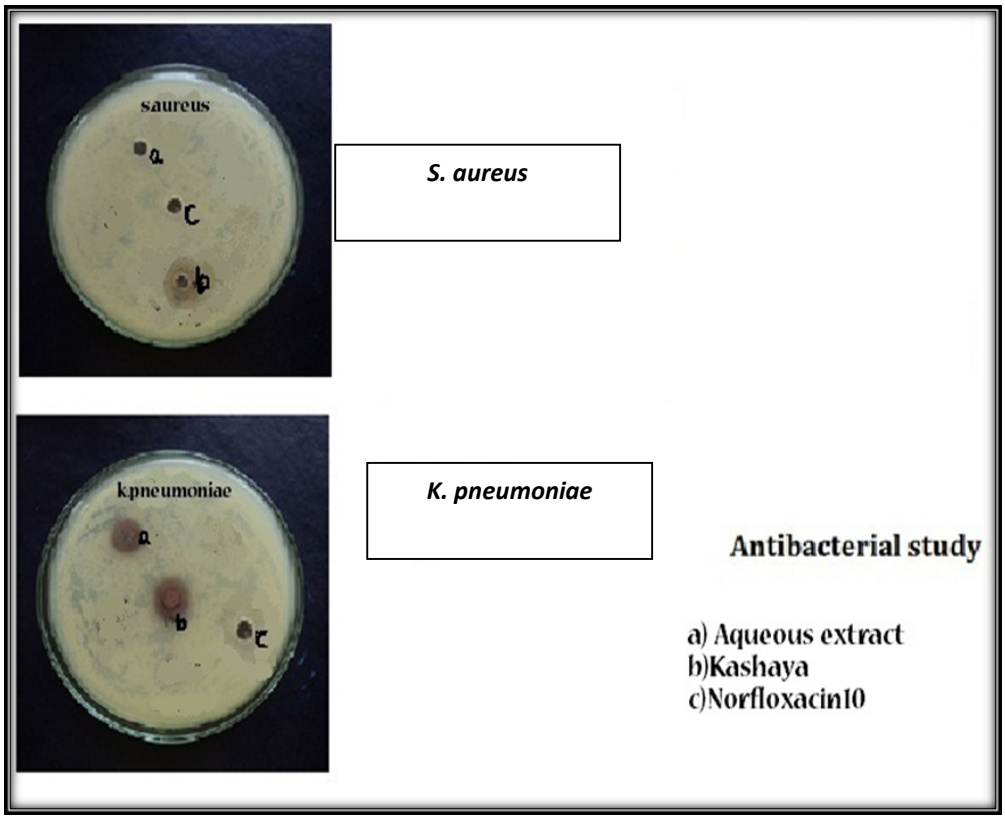

Fig. 3: Anti-Bacterial Study (Disk Diffusion Method)

4. CONCLUSION

Based on the Acute Oral Toxicological study which showed no signs of toxicity. Antibacterial study with Disk diffusion method showed the positive action of *Osbeckia muralis Kashaya* in *Klebseilla pneumoniae*.

5. ACKNOWLEDGMENTS

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