Research Article

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ANTI-MICROBIAL STUDY OF CALCINED SILVER (*RAJATA BHASMA*)

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ABSTRACT

RajataBhasma (RB) is a calcined silver compound widely used by practitioners of Ayurveda for various ailments like depletion of body elements, neurological disorders, muscular dystrophy, infertility, diabetes, various infectious conditions etc. It also lowers aggravated *Pitta Dosha* (humor responsible for metabolism and transformation) causing inflammatory condition. Previous studies have established pharmaceutical standardization and characterization of RB but Antimicrobial susceptibility study is not attempted yet. RB was prepared by *Kajjali* (Black Sulphide of Mercury) as a media and subjecting for *Laghu Puta*. (Incineration cycle with Max temp 550°C) Seven Repeated Calcination cycle was followed to achieve Chief Desired Characteristics (CDC). Further the end product was tested for its Classical accomplishment tests followed by physico-chemical analysis. Antimicrobial susceptibility was studied to understand the effectiveness of RB. RB was prepared in *7Putas* (Incineration cycle). All classical test for completion of the product were positive. Alcoholic extract of RB was found to be very effective against all the bacterial strains studied i.e. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with the diameter of zone of inhibition being 16, 22 and 22 mm respectively. RB showed moderate antibacterial activity by following disc diffusion method. However studies with different methods and solutions may be studied for better understanding of anti-microbial action of RB.

Keywords: Rasashastra, Kajjali, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*

INTRODUCTION

Rasashastra is a branch of Ayurveda explaining pharmaceutical aspects of conversion of metals and minerals into therapeutically potent drugs.¹ Among various metals described in *Rasashastra*, Silver is regarded as noble metal with vast therapeutic utilities since many centuries.² It is advised to use *Rajata* (Silver) in the form of *Bhasma* (Calcined formulation) for internal administration in single and multiple drug combinations. *Rajata Bhasma* (RB) (Calcined Silver compound) acts on depletion of body elements, neurological disorders, muscular dystrophy, infertility, diabetes etc. In various infectious conditions like fever, respiratory tract disorders, Urinary tract disorders etc. It also lowers aggravated *Pitta Dosha* (humor responsible for metabolism and transformation) causing inflammatory condition.³

There are few clinical studies carried out on RB particularly on hypoglycemic effect,⁴ free radical scavenging effect,⁵ etc. in post-graduation studies. Studies on different methods of preparation of RB have also been carried out² but no research work is done on antimicrobial activity of RB.

Several research works done by modern scientists regarding anti-microbial activity of silver compounds. Silver compound has shown effect on some bacteria, viruses, algae and fungi. Its germicidal effect kills many microorganisms in vitro. Antibacterial property of catalytic silver particles is utilized in filter candle for purification of water. Silver nanoparticles are

growing popularly in therapeutic field as the recent research works have indicated potential therapeutic value. Silver nano particles have been proved to be having anti proliferative activity,⁶ and most importantly bactericidal effect.⁷

Antimicrobial effect of Silver nanoparticles is well established and being applied successfully.⁸ RB is expected to produce Silver nano particles in a traditional method. Hence it is decided to evaluate RB for its anti-microbial effect. It is expected that the outcome of the study will substantially contribute to revalidate existing standardization with scientific evidences and to establish pharmacological properties of RB.

MATERIALS AND METHODS

Brief description regarding RB

Kajjali (Black sulphide of Mercury) was mixed with *Kumari Swarasa* (Aloe vera juice) to form paste consistency. Purified *Rajata* (Silver) foils were applied with this paste from both side and kept for drying. Further these foils were placed in *Sharava* (earthen plate) sealed and *Laghu Puta* (Incineration cycle with Max temp 550°C) was given. After first *Putra* (Incineration cycle), *Rajata* was in completely powder form. In subsequent *Putas*, equal amount of *Kajjali* to that of remnant was added and triturated with Aloe juice. Further *Chakrikas* (pellets) were prepared and *Putra* was given. Thus seven *Putas* were given to obtain RB which fulfilled all classical parameters. Cow dung cakes used for each *Laghu putra* were 20 in numbers and 2.5kg

weight. End product was ascertained for classical Chief Desired Characteristics (CDC) and Physico-chemical analysis. Further the sample of RB was subjected antimicrobial susceptibility

Bacterial cultures

The standard strains and antibiotic discs were obtained from Hi-Media, Mumbai, India.

Medium used in the disc diffusion technique was Muller-Hinton agar obtained Hi-Media, Mumbai, India.

Samples used

I. Test drug: RB suspended in following solvents

1. 30% alcohol
2. Honey with ghee in the ratio of 2:1
3. Distilled water

II. Positive controls:

1. Ampicillin (10µg/disc) for *Escherichia coli* ATCC 25922
2. Piperazillin (100µg/disc) for *Pseudomonas aeruginosa* ATCC 27853
3. Cefoxitin (30µg/disc) for *Staphylococcus aureus* ATCC 35923

III. Negative controls used were the discs soaked in solvents used for the preparation of suspension of RB viz, alcohol (30%), honey and ghee and distilled water respectively. The discs soaked in respective solvents were dried, placed on lawns and used as negative control.

Antibacterial activity using disc diffusion method

In the present study, sensitivity testing was done by Kirby-Bauer disc diffusion technique test pattern (NCCLS, 2002). Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antimicrobial discs were placed over the lawn and pressed slightly along with the positive and negative controls. Negative controls used were the discs soaked in solvents used for the preparation of suspension of RB viz, alcohol (30%), Ghee+Honey and distilled water. The discs soaked in respective solvents were dried, placed on lawns and used as negative control.

The plates were incubated for 18 hours at 37°C. The antimicrobial activity was evaluated for 5mg/disc and diameter of inhibition zone was measured. Experiment was carried out in triplicate and the average diameter of zone of inhibition was recorded.

Reading of zones of inhibition

The diameters of zones were measured to the nearest millimeter with calipers. The point of abrupt diminution of growth was taken as the zone edge.

Interpretation

Sensitive: Infection treatable with normal dosage

Intermediate: Infection that may respond to therapy with higher dosage or if the infection is in a situation where the agent is concentrated

Resistant: Not treatable with this agent

OBSERVATION & RESULTS

About 50% conversion Silver in to *Bhasma* was observed after two incineration cycles, however the product did not fulfil any desired characters. Further Incineration cycles were followed with *Kajjali* and *KumariSwarasa* till a perfect anticipated *Bhasma* was produced, which was seen after seven incineration cycles. (Table 1) Average temperature pattern observed during the incineration cycle is shown in Graph 1.

Alcoholic extract of RB was found to be very effective against all the bacterial strains studied i.e. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with the diameter of zone of inhibition being 16, 22 and 22 mm respectively. (Figure 1-3) This was comparable with the positive standards used. A mild antibacterial activity was observed when the drug RB was used as a suspension in honey. However, no activity against any of the microorganisms under test could be seen in aqueous extract of *Bhasma*. It was also observed that *Candida albicans* remained resistant against all the test drug samples used. (Figure 4) (Table 2)

Graph 1: Average temperature pattern followed during Incineration cycles

Table 1: Observations during preparation of RB

No of Incineration cycle	Wt. Before each Incineration cycle (g)	Wt. After each Incineration cycle (g)	Colour of Pellets (After incineration cycle)	Pellets on touch	Varitaratva*	Rekha Purnata**
1	170	120.4	Glistening silvery white particles on dark grey pellets	Cracks	-ve	-ve
2	250	105	Brownish black	Cracks Soft some pellets became powdered	-ve	-ve
3	210	99.4	Blackish gray	Easily breaks	-ve	+ve
4	200	72.2	Black	Breaks easily	-ve	-ve
5	147	68.2	Black along with reddish Spot in the middle	Breaks on Force	-ve	
6	136.5	66	Black	Soft	-ve	-ve
7	140	91.06	Black	Breaks on touch	+ve	+ve

*Varitaratva- *Bhasma* sprinkled over water surface (if it floats is said to be +ve), **Rekha Purnata- *Bhasma* rubbed between fingers will enter furrows

Table 2: Results of Antimicrobial susceptibility

Sl. No.	Organisms	Test drug	Zone size in mm	Sensitivity
1.	<i>Escherichia coli</i> ATCC 25922	Ampicillin (10µg/disc) (Positive control)	21	Sensitive
		RB in alcohol (30%)	16	Sensitive
		RB in honey and ghee	8	Sensitive
		RB in distilled water	6	Resistant
2.	<i>Pseudomonas aeruginosa</i> ATCC 27853	Piperazillin (100µg/disc) (Positive control)	30	Sensitive
		RB in alcohol (30%)	22	Sensitive
		RB in honey and ghee	8	Sensitive
		RB in distilled water	6	Resistant
3.	<i>Staphylococcus aureus</i> ATCC 35923	Cefoxitin (30µg/disc) (Positive control)	29	Sensitive
		RB in alcohol (30%)	22	Sensitive
		RB in honey and ghee	8	Sensitive
		RB in distilled water	6	Resistant
4.	<i>Candida albicans</i>	RB in alcohol (30%)	6	Resistant
		RB in honey and ghee	6	Resistant
		RB in distilled water	6	Resistant

Figure 1: Activity of test drugs against *Escherichia coli* Figure 2: Activity of test drugs against *Pseudomonas aeruginosa* Figure 3: Activity of test drugs against *Staphylococcus aureus* Figure 4: Activity of test drugs against *Candida albicans*

DISCUSSION

RB was prepared in seven incineration cycles in current experiment, however by following different references the number of cycles may increase or decrease. In one of the studies RB was prepared in 4 incineration cycles but in other experiment it took 11 & 17 cycles.^{2,9} This purely depends on media selected for incineration method adopted and the quantity of *Rajata* taken for incineration.¹⁰ However the end point of all the experiments were determined by *Bhasma Pareeksha*. (Chief Desired Characteristics) RB was found to be black coloured smooth lusterless state due to transformation into compound form after incineration cycles. The gradual transformation of metal to compound form takes place due to the process followed during each incineration cycle. In each of this process HgS Black sulphide of mercury is added as media. The whole mixture is triturated with *Aloe vera* juice, this step facilitates uniform mixture of media with the silver. Then the resultant

mass of trituration is made in to *Chakrika* (pallet) to provide maximum surface area to react. Then these pellets are placed, sealed in earthen plates and incinerated. Oxidation reaction taking place during incineration with temperature 500°C and above will ensure the end product is free from mercury. Several studies of *Bhasma* prepared by mercury as media showed absence of the same in the end product. At the same time the silver which has affinity towards sulphur will react to form a compound. Black sulphide of mercury not only acts as media to enhance the reaction and compound formation but also yields therapeutic potential to the end product.

The problem encountered during the evaluation of RB for antimicrobial susceptibility was related with its solubility. Silver sulfide a major compound in the product is almost insoluble in water, the solubility being as low as 8.5-12 mg/L. Hence no activity could be appreciated. It was sparingly soluble in alcohol. Honey and ghee were used because they are the main

Anupana (Adjuvant) used for RB. Even though alcohol, honey and ghee have antimicrobial potential, the activity of RB samples in these media were confirmed after comparing with the negative control where these media were used. However, preparation of a uniform suspension in water with the help of different suspending agents may have to be tried to get clear information.

In vitro studies have proved that the bioactive silver ion (Ag⁺) in adequate concentration can kills bacteria. External wounds infected by bacteria in living tissue can be nullified with silver ions.¹¹ Silver compounds containing biologically active silver ion (Ag⁺) can act as antibacterial based on its capability to damage key enzyme systems irreversibly in the cell membranes of pathogens.¹² Silver nanoparticles formed by Metallic silver has a remarkable antimicrobial potential.¹³ Ag nanoparticle was investigated for antimicrobial activity and found that, Ag nanoparticles inhibited growth of *Yeast* and *E. coli*, whereas on *S. aureus* the inhibitory effects were mild.¹⁴ Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.¹⁴

CONCLUSION

RB showed moderate antibacterial activity by following disc diffusion method. The present study has shown encouraging results to traditionally prepare calcined silver preparation. However, further study by rectifying certain areas such as solubility and different study patterns in antimicrobial evaluation may lead to consolidated outcome.

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