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Bithika Halder
M.V.Sc. Scholar, Department of Veterinary Medicine, Ethics and Jurisprudence, W.B.U.A.F.S., Kolkata, West Bengal, India

Samar Sarkar
Professor, Department of Veterinary Medicine, Ethics and Jurisprudence, W.B.U.A.F.S., Kolkata, West Bengal, India

Samiran Bandapadhyay
Senior Scientist, Indian Veterinary Research Institute (IVRI), Kolkata, West Bengal, India

Prasanta Kumar Sarkar
Professor and Head, Department of Rasashastra, J.B. Roy State Ayurvedic Medical College and Hospital, Kolkata, West Bengal, India

Amit Raj Gupta
Associate Professor and Head, Department of Veterinary Medicine, Ethics and Jurisprudence, W.B.U.A.F.S., Kolkata, West Bengal, India

Subhasish Batabyal
Professor and Head, Department of Veterinary Biochemistry, W.B.U.A.F.S., Kolkata, West Bengal, India

Corresponding Author
Bithika Halder
M.V.Sc. Scholar, Department of Veterinary Medicine, Ethics and Jurisprudence, W.B.U.A.F.S., Kolkata, West Bengal, India

Evaluation of selected ayurvedic nanoparticle drug Rasamanikya against MDR pathogen in mice model

Bithika Halder, Samar Sarkar, Samiran Bandapadhyay, Prasanta Kumar Sarkar, Amit Raj Gupta and Subhasish Batabyal

Abstract
This study look for the efficacy of selective ayurvedic nanoparticle drug against bacterial infection in murine model. As the antimicrobials are rapidly losing their efficacy, it is urgent to further our research for new and effective alternative to combat such multi-drug resistant isolates. Medicine consists nanoparticles known as nanomedicine which desires deliver research tools and clinically reformative devices in the near future for having many advantages such as specific drug delivery system, reduced toxic effects while continuing therapeutic effects, biocompatible, faster and safe medicine. Nanoparticle drug containing particles ranges from 1 to 100 nm in size, an application of nanotechnology which made its debut with greatly increased possibilities in the field of medicine. In the present study ayurvedic herbo-metallic drug which is reported as nanoparticle drug, Rasamanikya was evaluated against the ESBL producing/carbapenem resistant *E. coli* bacterial infection in mice model. Rasamanikya is used in powder form at 16.25 mg/kg dose in mice. One microlitre of bacteria (corresponding to 1×10^7 or 1×10^8 CFU for immunocompetent mice and 1×10^3 or 1×10^5 CFU for immunocompromised mice) with normal saline solution was inoculated subcutaneously to produce infection. On the next day skin lesion was developed in the inoculated site and the mice was treated with Rasamanikya @ 16.25 mg/kg dose orally twice daily for 7 days with vehicle (honey). The drug was also used for antiseptic therapy on the lesion by mixing with soft paraffin in 1:1 ratio twice daily. After 7 days of the treatment the lesion was almost healed and the mice was recovered. For haemato-biochemical test blood sample was collected by heart puncturing method and tissue sample were collected from the mice to investigate the histopathological changes after sacrificing of animal by cervical dislocation.

Keywords: MDR pathogen, ayurvedic nanoparticle drug, Rasamanikya, mice, antibacterial and antiseptic efficacy

1. Introduction
In the present era, huge amount of antibiotics used for human as well as animals therapy resulting in resistance of microorganisms to the administered antimicrobial medicines despite earlier sensitivity to it, known as Multidrug resistance (MDR) pathogens [1, 2]. As the MDR is an increasing public health concern worldwide, it is urgent to further our research for new and effective alternative to combat such multi-drug resistant isolates [3, 4]. Nanoparticles are small scale substances (<100 nm) whose exposure is rising among humans and animals due to the increase in their applications [5, 6]. Many nanoparticle shows potential antibacterial activity against various Gram + ve and Gram -ve bacteria including highly methicillin and carbapenem resistant strains. Nanotechnology is a fast-growing technology that plays an important great impact on various fields of therapeutic applications and capable for solving several problems related to animal health and production [7] with the principle to prevent the adverse effects of loaded drugs by reducing the amount of drugs. On the other hand, ayurvedic system of medicine is one of the oldest systems of Indian traditional medicine mostly based on metal-mineral formulation commonly known as Bhashma having particle in a diameter of about 10-15 nm [8] and they renovated review on scientific literature on various types of Bhashma and their therapeutic efficacy along with metal nanoparticle. Combination of ayurveda and nanotechnology may provide the best solution as a medicine to treat various life-threatening diseases [9]. Mineral rich Rasamanikya Nanoparticle (RMNP) which is ayurvedic herbo-metallic nanomedicine have antimicrobial and anticancer potential assessed by *in-vitro* cellular assay [10]. It has been commonly used in various skin diseases, bronchial asthama, eczema, fistula, gout and syphillis [11]. So the present study was conducted for evaluation of RMNP and to investigate the effects of this drug administration on haemato-biochemical parameters and histopathological features.

2. Materials and Method

The study is conducted for evaluation of antibacterial and antiseptic efficacy of Rasamanikya reported as ayurvedic herbo-metallic nanoparticle drug [10] against the ESBL Producing/carbapenem resistant *E. coli* bacterial infection in mice model. *In-vitro* and *in-vivo* both are performed in this case. Rasamanikya was collected from Dept. of Rasashastra of J.B. Roy State Ayurvedic Medical College and Hospital prepared by Dr. P.K. Sarkar, Professor and head of the department.

2.1 *In vitro* study

The *in-vitro* experiment for assessment of antimicrobial sensitivity profile against MDR bacterial strains of E. coli was performed by following two methods i.e. plate diffusion and disc diffusion in the laboratory of Indian Veterinary Research Institute (IVRI), Kolkata in West Bengal state under the supervision of Dr. Samiran Bandapadhyay, senior scientist of Veterinary Microbiology department.

2.1.1. Plate diffusion method

250 ml Muller-Hinton Agar media is mixed with different concentration of Rasamanikya i.e. 2.5 mg, 5 mg and 10 mg. The media was placed into sterilized petriplates and leave it to solidify. 1 micrlitre of 0.5 MacFarlane Bacterial culture were placed into that petriplates and kept in incubator for overnight after drying.

2.1.2. Disc diffusion method

Muller-Hinton Agar medium was prepared, sterilized and poured into the sterile petriplates and was allowed to solidify. By using cotton swab bacterial culture was uniformly spread on to the plates containing the media. To make suspension 100 mg of Rasamanikya was dissolved in 250 microliters of Methanol and 500 microliters distilled water. By using Micropipette measured volume of suspension were taken in each disc to make Different concentration i.e. 2.5 mg, 5 mg, 10 mg for evaluation of zone of inhibition. After drying the discs were placed on the previously swabed petriplates and kept in incubator for overnight.

2.2 *In vivo* study

The Institutional Ethical Committee (IAEC) approved the experimental protocol to conduct the study in mice model considering the inclusion and exclusion criteria. The mice were fed with balanced pelleted diet and maintained under strict laboratory conditions, controlled with environmental, temperature, humidity and light dark cycles. After 21 days of maintenance the fur will be removed from the back of the mouse using clippers, One microlitre of bacteria (corresponding to 1 $\times 10^7$ or 1 $\times$ 10^8 CFU for immunocompetent mice and 1 $\times 10^3$ or 1 $\times$ 10^5 CFU for immunocompromised mice) with normal saline solution was inoculated subcutaneously to produce infection (Fig 3). On the next day lesion was developed in the inoculated site and the treatment was started with powder form of Rasamanikya @ 16.25 mg/Kg [12] orally twice daily for 7 days with vehicle (honey). The drug was also used for topical application with soft paraffin in 1:1 ratio (Fig 5). After one week of treatment the lesion was almost healed and the mice was recovered. Blood sample was collected by heart puncturing to see haemato-biochemical changes i.e. Hb%, SGPT, SGOT, ALP, Urea and Creatinine and and tissue samples were collected from the mice to examine the histological changes for evaluating the toxic effect of trialed drug after sacrificing of animal by cervical dislocation. The haemoglobin percentage was estimated by using colorimeter and the biochemical tests were performed by using Chem. 5x model machine (Transition Biomedical Limited) in the biochemistry laboratory, W.B.U.A.F.S., Kolkata (West Bengal) (Fig 8). For histopathological examination liver and kidneys were collected and the tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin (H&E) for light microscopic examination.

3. Result and Discussion

The main intention of conducting this study was to find out and understand various effects produced by the drug Rasamanikya. There are several studies which are able to evaluate the efficiency and toxicity of the drug Rasamanikya [10, 13]. In the current study Rasamanikya was able to inhibit the bacterial colony growth on 5 mg and 10 mg concentration in plate diffusion method and the strains was taken for further study to evaluate the zone of inhibition (ZOI) by disc diffusion method. It appears from the study that RM has some antibacterial effect against the ESBL producing/ carbapenem resistant *E. coli* of animal origin, though it effect was evident in disc diffusion test (Table 1., Fig 2). It appears from the study that RM has some antibacterial effect against the ESBL producing *E. coli* of animal origin, though it effect was only evident in disc diffusion test. After inoculation of bacterial solutions subcutaneously lesion were produced in the inoculated region on next day (Fig 4) and treatment was started immediately. Drug was given in both way orally and topically. After one week of treatment the lesion was almost healed and the animal was successfully recovered (Fig 6). Haemato-biochemical parameters were not altered to significant level by the drug treatment (Table 2). Before therapeutic application of these compounds in Ayurveda, purification treatment is done. And these drugs are prescribed mixing with specific vehicle according to infection. Then only these drugs are used as therapeutic entity. And at therapeutic dose these drugs are found to be safe. Their use in Skin diseases (Kushtha), leprosy gives an indication that the drug is effective in bacterial diseases, as well. Several studies are conducted to evaluate the efficiency and toxicity of the drug Rasamanikya In this study, the test drugs were prepared following the Ayurvedic procedures after proper purification treatment. And the drug shows no toxic effect in murine model.

Table 1: Result of disc diffusions method

Isolates name	Blank (mm)	2.5 mg/ml (mm)	5 mg/ml (mm)	10 mg/ml (mm)
SL11a (*E. coli* strain)	6	17	19	23

Table 2: Haemato-biochemical parameters of experimental mice

Parameters	Normal/Healthy Animal*	Experimental Animal
Haemoglobin % (mg/dL)	13-16	13.4
SGPT/ALT (U/L)	17-77	33.0
SGOT/AST (U/L)	54-298	78.0
ALP (U/L)	35-96	46.0
Urea (mg/dL)	15-59	32.0
Creatinine (mg/dL)	0.2-0.9	0.6

*Reference value source: wikivet. And Modified from Loeb *et al.* (1999)

Fig 1: Ayurvedic herbo-metallic nanoparticle drug Rasamanikya

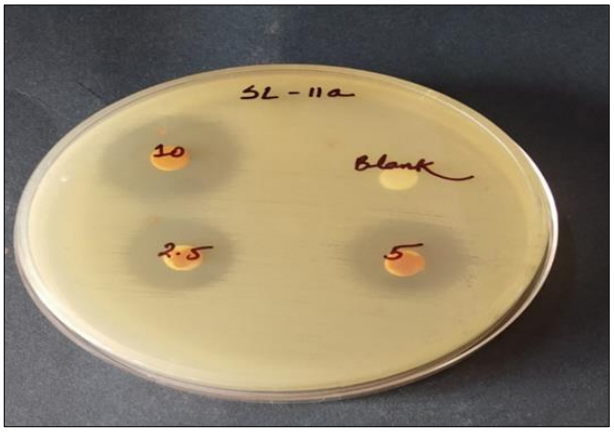

Fig 2: Disc diffusion method showing Zone Of Inhibition

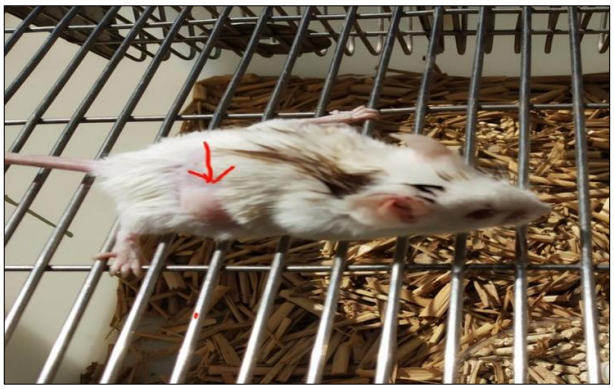

Fig 3: MDR bacterial strains subcutaneously inoculated in mice

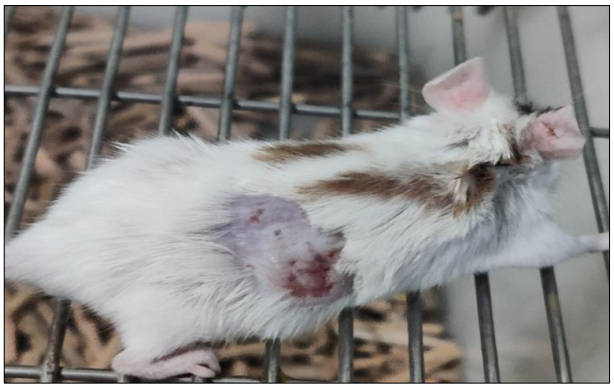

Fig 4: Infection develops by inoculation of pathogen (*E. coli* strain)

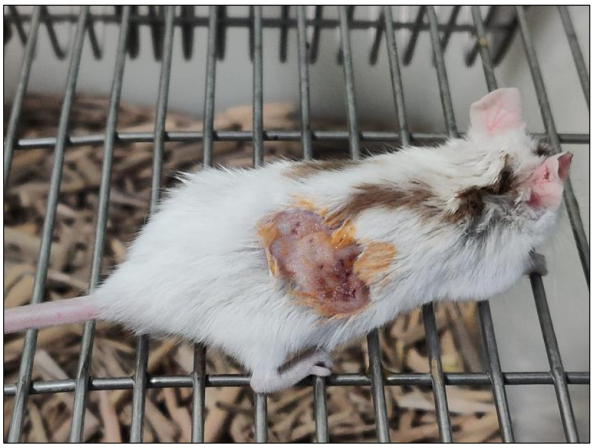

Fig 5: Topical application of Rasamanikya with soft paraffin

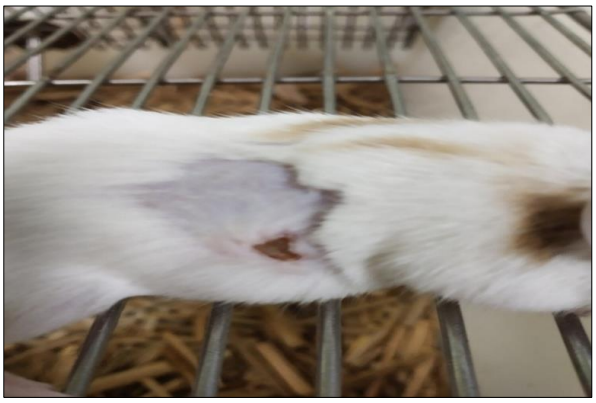

Fig 6: Healing of lesion within 7 days

Fig 7: Evaluation of haemato-biochemical parameters

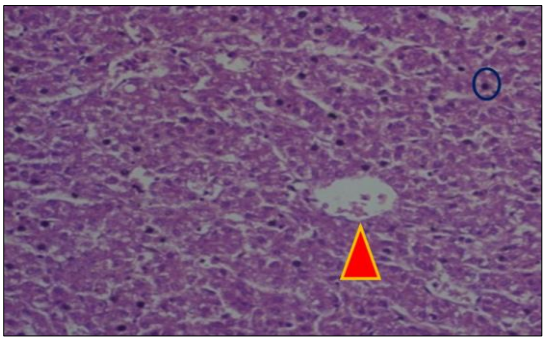

Fig 8: Histological features of liver after treatment with Rasamanikya a) orange arrow represents central vein, b) Blue circle represents hepatic cells

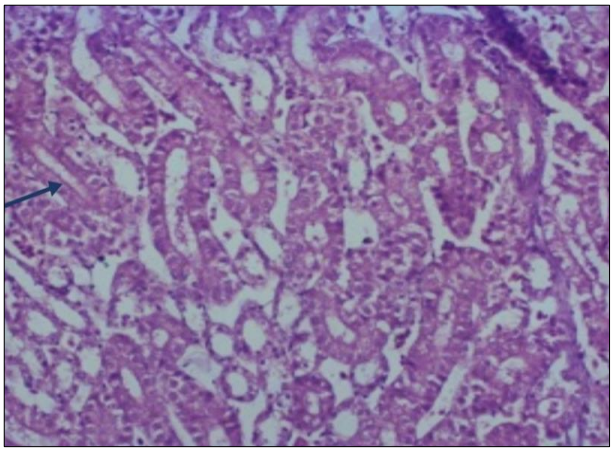

Fig 9: Histological features of kidney after treatment with Rasamanikya a) Blue arrow represents glomerular cells

4. Conclusion

It can be concluded that ayurvedic herbo-metallic nanoparticle drug Rasamanikya has antibacterial and antiseptic efficacy against ESBL producing/ carbapenem resistant *E*. coli in both *in-vitro* and *in-vivo* assessment and It showed no adverse effects in histological and haemato-biochemical parameters in murine model.

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