The Haryana Veterinarian (June, 2023) **62(1),** 5-7 ISSN 0033-4359 (P) **Research Article**

IN VITRO ANTIMICROBIAL ACTIVITY OF PIPERINE AGAINST MULTI-DRUG RESISTANT *SALMONELLA* SPP.

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ABSTRACT

Non-typhoidal salmonellosis is the leading cause of bacterial gastroenteritis that causes significant morbidity and mortality worldwide. Poultry and poultry products serve as the most important source of this infection to humans. With the reduced antimicrobial discovery pipeline, current studies are focused on the exploration and utilization of phytochemical approaches. Therefore, the present study was undertaken to investigate the *in vitro* antimicrobial activity of piperine against multi-drug resistant *Salmonella* spp. (*S.* Enteritidis and *S.* Typhimurium). Piperine exhibited excellent antibacterial efficacy against the tested pathogens with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.781 and 3.125 µg/ml, respectively. Overall, the study demonstrated that piperine could be explored as a potential antibiotic alternative against drug-resistant pathogens.

Keywords: Alternatives, Antimicrobial Resistance, Non-typhoidal *Salmonella*, Piperine

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The discovery and commercialization of antibiotics have revolutionized modern medicine and science creating a substantial change in the therapeutic paradigm of infectious diseases. However, the global emergence and spread of antibiotic resistance are pre-eminent public health menace of the 21st century, owing to their profound effect on humans, animals, and the environment (Mamun *et al.*, 2021). Furthermore, the indiscriminate use of antibiotics continues to rise, although the novel antibiotic discovery is at a limited pace. Non-typhoidal *Salmonella* (NTS) is considered the foremost cause of bacterial gastroenteritis worldwide, leading to life-threatening complications depending upon host immunity. Moreover, drug-resistant NTS serotypes have been categorized as a 'serious' threat posing public health jeopardy by Centers for Disease Control and Prevention (CDC, 2019).

The dwindling antibiotic discovery pipeline has shifted the research paradigm, leading to the development of novel strategies to combat AMR. Of late, there is a growing interest in developing and designing plant-based pharmaceuticals due to their potential to fight against drug-resistant pathogens (Shityakov *et al.*, 2019). Piperine, a pungent alkaloid found in the seeds of black pepper (*Piper nigrum* L.) is a novel candidate for the development of various bioactive compounds. Piperine and its derivatives possess promising therapeutic potential owing to its antimicrobial, anti-inflammatory and anti-cancerous properties. The objective of the present study was to investigate the *in vitro* antimicrobial efficacy of piperine against the multi-drug resistant (MDR)- NTS strains, especially the *S.* Typhimurium and *S.* Enteritidis.

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MATERIALS AND METHODS

Literature survey: A google scholar- based literature survey was initially conducted for evaluating the antimicrobial properties of piperine, an alkaloid found in plants belonging to the Piperaceae family, especially black pepper (*Piper nigrum* L.) often termed as the 'King of Spices' (Amperayani *et al.*, 2018). Piperine has been identified to be the major bioactive component present in black pepper and displays numerous therapeutic benefits including antimicrobial, antihypertensive, antioxidant, analgesic, anti-diarrhoeal, antidepressant, antiplatelet, and anti-cancerous properties. In addition, the previous studies demonstrated the inhibitory action of piperine against various MDR pathogens including methicillin-resistant *Staphylococcus aureus*, *S.* Typhi, *Escherichia coli* and *Proteus* spp. (Amperayani *et al.*, 2018; Khare *et al.*, 2021).

Strains used: The characterized MDR field strains of *S.* Enteritidis (S1; S2; S3) and *S.* Typhimurium (ST1; ST2; ST3) maintained in the laboratory repository of the Department of Veterinary Public Health at College of Veterinary and Animal Sciences, Pookode were used to evaluate the *in vitro* antibacterial efficacy of piperine.

***In vitro* antimicrobial activity of Piperine:** To evaluate the *in vitro* antimicrobial efficacy of piperine, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined against the characterized field strains (*S.* Typhimurium and *S.* Enteritidis) by micro broth dilution technique (CLSI, 2019). Piperine (Sigma Aldrich, USA) was prepared to a final concentration of 10 mg/ml (stock solution) in 100% ethanol.

In brief, the MIC was determined by co-incubating 100 µl of the individual test cultures (at a final concentration of 1×10^7 CFU/mL) with decreasing concentrations of 1 percent piperine (25 to 0.195 µg/ mL) in 100 µl of cation-adjusted Mueller Hinton broth (CA-MH; HiMedia Laboratories Pvt. Ltd., Mumbai, India) in a 96- well flat-bottom microtiter plate. After the incubation at 37ºC for 18-24 h, resazurin dye (0.015%) was added to all the wells to determine the dye reduction (from purple to pink) and thereby the bacterial inhibition.

The lowest concentration of piperine without visible growth was designated as MIC, while the MBC of piperine was estimated by plating 10 µl aliquots drawn from each well (Miles *et al.*, 1938) revealing no visible growth in Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India). The lowest concentration of the piperine which revealed 99.90% killing of test cultures in the XLD agar was determined to be its MBC (NCCLS, 1999).

RESULTS AND DISCUSSION

In general, NTS is recognized as one of the most common bacterial causes of food-borne gastroenteritis worldwide. Poultry and poultry products are often considered as the important risk factor responsible for the transmission of salmonellosis to humans (Sarker *et al.*, 2021). However, the indiscriminate use of antimicrobials against highly invasive subtypes of NTS has resulted in the emergence of multidrug resistance further reducing the efficacy of currently available antibiotics (Gupta *et al.*, 2020). Hence, there is an urgent need for novel intervention strategies to tackle the burgeoning issue of AMR.

Piperine is the major bioactive component and naturally occurring alkaloid isolated from *P. nigrum* L, which is extensively distributed in India (Hegeto *et al.*, 2019). It possesses excellent antioxidant, antimicrobial, anticonvulsant, larvicidal, antiparasitic, neuroprotective, anticancer effects, and other pharmacological properties (Quijia and Chorilli, 2020).

Table 1. MIC and MBC values of piperine

	Isolates	MIC (µg/ ml)	MBC (µg/ ml)
S. Enteritidis	S1	0.781	3.125
	S2	1.5625	6.25
	S3	0.781	3.125
S. Typhimurium	ST1	0.781	3.125
	ST2	0.781	3.125
	ST3	0.781	3.125

***In vitro* antimicrobial activity of Piperine:** The results of MIC and MBC values of piperine determined against the characterized field strains of *S.* Typhimurium and *S.* Enteritidis are presented in Table 1. The results suggest that piperine could serve as a potential antimicrobial candidate against MDR field strains of non-typhoidal *Salmonella* spp.

Piperine proved to be efficient against various bacterial pathogens including *S. enterica* serovars and *S. epidermidis* (Moraru *et al.*, 2019). It has been suggested that piperine improves the production of interleukin- 6 and enhances amino acid transporter, thereby promoting amino acid metabolism and further increasing the production of necrosis factors and cytokines in bacterial cells (Haq *et al.*, 2021). In contrast to the present study, Tokam Kuate *et al.* (2021) have examined the antimicrobial effect of piperine and demonstrated that it is less effective against *S. enterica* serovars including *S.* Typhi, *S.* Typhimurium, *S.* Enteritidis and *S.* Choleraesuis. Despite the various therapeutic benefits of piperine, the low solubility in aqueous media and poor bioavailability serves as a barrier for its biomedical applications and development as a drug in the future (Shityakov *et al.*, 2019; Quijia and Chorilli, 2020).

CONCLUSION

The present study investigated the *in vitro* antimicrobial activity of piperine against the MDR field strains of *S.* Typhimurium and *S.* Enteritidis. As demonstrated by the micro broth technique, piperine exhibited excellent antimicrobial activity against the NTS isolates. However, further studies need to be undertaken for determining the safety and stability parameters of piperine before its clinical translation in suitable target hosts. Furthermore, novel strategies should be adopted to improve its biomedical application by incorporating into different drug delivery systems and combination therapies

with currently available antibiotics.

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