Haryana Vet. (June, 2023) **62(1),** 1-4 ISSN 0033-4359 (P) **Research Article**

EFFECT OF FENUGREEK (*METHI*) SUPPLEMENTATION ON MILK PRODUCTION, MILK COMPOSITION AND HAEMATO-BIOCHEMICAL PARAMETERS IN MURRAH BUFFALOES

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Received: 17.02.2022; Accepted: 19.05.2022

ABSTRACT

The envisaged research was undertaken to study the effect of Fenugreek (*Methi*) supplementation on milk production, milk composition and haemato-biochemical parameters in Murrah buffalo. In this study, eighteen lactating Murrah buffaloes were randomly divided into three treatment groups having six animals in each on the basis of milk yield, body weight and parity. Basal roughage-concentrate mixture diets as per ICAR (2013) feeding standards were provided to the buffaloes in the control group (T_1). The experimental treatment groups included basal ration (control) supplemented with crushed fenugreek seeds @150 g per animal per day (T_2) and basal ration (control) supplemented with crushed fenugreek seeds @ 250 g per animal per day (T_3). The experimental animals were housed under loose housing system following standard managemental practices and biosecurity measures. The experimental period was from 10th day postpartum to completion of 6th month of lactation of all the experimental animals. The results of present investigation revealed that supplementation of fenugreek (*Methi*) have significant effect on serum glucose and serum cholesterol levels in lactating Murrah buffaloes. Also, fenugreek (*Methi*) supplementation did not have any adverse effect on average daily milk yield and milk composition i.e. milk fat, 6% FCM, milk protein, SNF content, total solids and lactose in lactating Murrah buffaloes upto six months of lactation.

Keywords: Fenugreek, Murrah, Milk yield, Milk composition, Biochemical parameters

How to cite: Sarowa, A., Chhikara, S.K., Sahu, S., Yadav, D.C., Amandeep, Ghotar, R. and Komal (2023). Effect of fenugreek (*methi*) supplementation on milk production, milk composition and haemato-biochemical parameters in murrah buffaloes. *Haryana Vet.* **62(1)**: 1-4.

The use of herbal galactogogues is known to have beneficial effect on milk production. Herbs are safe to use, cheap and easily available, has no side effect and no residual effect in milk in long term. Fenugreek (*Trigonella foenum graecum*), locally known as *Methi*, is one of the oldest herbs which grows throughout India and other parts of the world. Fenugreek (*Trigonella foenum-graecum*) is derived from a plant that belongs to the leguminous family. It is commonly known as *Methi* and is found in India, Middle East, North Africa and South Europe. The seeds are highly valued as food for man, cattle and sheep and to promote lactation and lactation performance in woman and ruminant. Fenugreek has been shown to have a positive effect on lactation performance in ruminants such as dairy cows, buffaloes and dairy goats (Nasser *et al.*, 2013; EL-Basheir 2015; Degirmencioglu *et al.*, 2016).

The World Health Organization encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature. Herbs are safe to use, cheap and easily available, has no side effect and no residual effect in milk in long term. The seeds of fenugreek are known to have hypoglycaemic, hypocholesterolemic, gastro and hepato-protective and anti-oxidant properties. Fenugreek seeds also lowered serum triglycerides, total cholesterol and low-density lipoprotein cholesterol.

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MATERIALS AND METHOD

In the present study, eighteen lactating Murrah buffaloes were selected and divided into three treatment groups having six in each on the basis of milk yield and body weight and parity following completely randomized design (CRD) from the herd of buffalo farm, Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. All the experimental lactating Murrah buffaloes used in the present study were kept under loose housing system. Prior approval was taken to conduct the present investigation from the Institutional Animal Ethics Committee. The daily allowance of concentrate mixture was offered to each animal at the time of milking in the morning and evening as per the Experimental diet *i.e.* Treatment 1 (control): Basal roughage-concentrate mixture diets as per ICAR (2013) feeding standards (T_1), Treatment 2: Basal ration (control) supplemented with crushed fenugreek seeds @150 g per animal per day (T_2) and Treatment 3: supplemented group with crushed fenugreek seeds @ 250 g per animal per day (T_3). All standard managemental practices and biosecurity measures were followed throughout the experiment. Prior to start of the experiment an adjustment period of 10 days was given to all the experimental buffaloes for acclimatization to the new house and feeding regimen. The experimental buffaloes were daily washed before milking. The animals

were maintained under isomanagerial conditions and similar husbandry practices except the different feeding treatments. The study was conducted for a period from 10 days postpartum to 6 months of lactation. Before formulation of rations, the feed ingredients were analyzed for proximate composition (Table 1). Buffaloes were hand milked twice daily and milk yield was recorded by using digital weighing balance and the weekly average was calculated. Milk sample for composition analysis were first taken at tenth day after calving and thereafter at each fortnightly interval until the end of the experiment. These samples were taken during the morning and evening milking from each experimental buffalo and analysed with the help of Milko-Scan autoanalyser and the average of both times was calculated for estimation of milk quality in terms of Milk fat (%), Milk protein (%), Solid not fat content(%), Total solids (%) and 6% FCM = 0.308 × Total milk + 11.54 × Total fat.

From all the eighteen buffaloes belonging to the three experimental treatments, blood samples were collected aseptically during early morning hours before feeding and watering of buffaloes by jugular vein puncture. Approximately ten millilitre (ml) of blood was collected from each animal and transferred immediately into a set of sterile plastic tubes without anticoagulant. The contents of test tubes were held in slanting position for serum separation. The sera were centrifuged to remove the erythrocytes present, if any. The clear, non haemolysed sera were then collected in clean, dry and labelled vials. These sera were preserved under deep freezing in capped vials for further analysis. Blood sample were collected at the begining of the experiments i.e. day 0, and thereafter at monthly intervals that are on day 30, 60, 90, 120, 150 and 180th of experiment periods. The sera samples were analysed for estimation of biochemical parameters namely Blood glucose, Serum calcium, Serum cholesterol, Serum total protein, Serum globulin, Serum albumin, Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) using Automated Random Access Clinical Chemistry Analyser (EM Destiny 200) TM, Erba Diagnostics Mannheim GmbH). All the data were subjected to ANOVA using General Linear Model's procedure of SPSS-23 software (SPSS, 2019). The mean differences among different treatments were separated by Duncan's multiple range tests. Consequently, a level of ($P<0.05$) was used as the criterion for statistical significance (Duncan, 1955).

RESULTS AND DISCUSSION

Daily milk yield

The overall mean values of daily milk yield were 11.21, 11.11 and 11.46 in treatment groups T_1, T_2 and T_3, respectively. The result showed statistically no significant difference between treatments except at seventh week where mean values are higher in T_3 (Table 2 & Fig. 1). Kirar *et al.* (2018) reported that supplementation of fenugreek seeds @50 g and 100 g were accompanied by an increase in the daily milk yield and 90 days total milk yield compared to control group, but the increase was not significant in lactating Murrah buffalo. Trian (2003) suggested that increase in milk yield in fenugreek supplemented groups compared to control group might be due to the effect of diosgenin, which is a chemical compound in fenugreek seeds similar to the hormone oestrogen stimulating an increase in milk flow. Over a period of 180 days of experiment it was observed that the supplementation of fenugreek (*methi*) @ 150 g and 250 g did not have significant effect on average daily milk yield of the experimental buffaloes. Abo El-Nor *et al.* (2007) reported that supplementation of 200 g fenugreek seeds resulted in significant ($P<0.05$) increase in milk yield in lactating buffaloes. Maher and Nadya (2012) reported that feeding of 2 or 4% of Fenugreek seeds in Friesian cows and Nasser *et al.* (2013) reported that supplementation of 50 g and 100 g of fenugreek seed powder per cow per day in Sharabi cows significantly ($P< 0.05$) increased milk yield. Degirmencioglu *et al.* (2016) also reported that supplementation of 50 g ground fenugreek seeds significantly ($P<0.01$) increased mean daily milk production in Anatolian water buffaloes.

Milk composition

The overall average milk fat (%) in T_1,T_2 and T_3 groups were 6.93 ± 0.07, 7.07 ± 0.05 and 7.09 ± 0.05, respectively (Table 3). The overall average FCM (kg) in the three treatment groups were 12.14, 12.24 and 12.60 kg, respectively. The supplementation of fenugreek (*Methi*) @ 150 g and 250 g showed non-significant effect on milk composition in terms of milk fat, FCM, milk protein, SNF content, total solids and lactose and at each progressive sampling during the experimental period. Shah and Mir (2004) and El- Alamy *et al.* (2001) through their experiments found that feeding fenugreek seeds to buffaloes had no effects on milk composition. Kholif and Abdel-Gawad (2001) also reported that there was no significant difference on milk lactose content in fenugreek supplemented group compared to control group. Balgees *et al.* (2013) similarly reported that milk components (protein, lactose and SNF) showed inconsistent pattern by fenugreek seeds supplementation but there was significant ($P<0.05$) decrease in milk fat percentage. EL-Basheir (2015) also reported that supplementation of fenugreek Seeds @ 0, 2.5and 5% of the diet did not affect milk

Table 1. Proximate composition (%DM basis) of different feed ingredients fed to the experimental buffaloes

Ingredients	DM	CP	CF	EE	Ash	OM	NFE
Wheat straw	94.56	2.42	35.91	2.85	11.31	88.69	47.51
Berseem (Green)	22.95	15.73	27.51	3.11	8.55	91.45	53.1
Maize (Green)	24.91	10.43	26.2	4.29	11.14	88.86	47.94
Maize	89.02	9.17	2.31	3.65	1.08	98.92	83.79
Barley	93.49	9.76	7.89	1.81	4.83	95.17	75.71
Groundnut cake	95.56	45.71	8.65	6.29	5.15	94.85	34.2
Soyabean meal	89.45	45.91	4.69	2.93	7.37	92.63	39.1
Mustard cake	94.37	32.21	7.98	6.65	7.81	91.26	45.35
Fenugreek seed	89.81	28.40	9.30	7.14	3.28	96.72	51.88

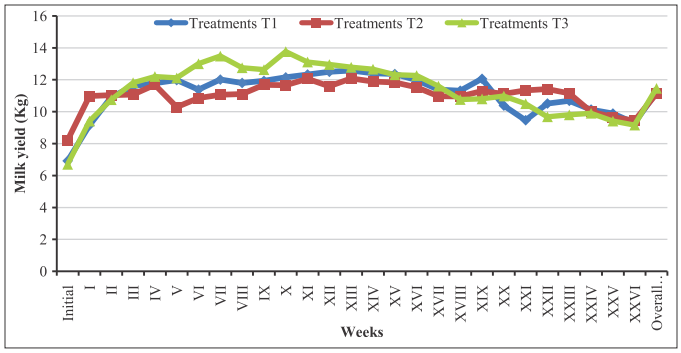

Fig. 1. Average milk yield (kg) of experimental buffaloes at weekly intervals under different dietary treatments

composition. Degirmencioglu *et al.* (2016) also reported that supplementation of 50 g of ground fenugreek seeds in Anatolian water buffaloes had no significant effect on SNF and protein percentages in milk. Contrary to the findings of the present study, Nasser *et al.* (2013) reported that supplemented fenugreek seed powder at a level 50 or 100 g/cow/day in Sharabi dairy cows resulted in significantly (P 0.05) increased milk fat percentage.

Haemato-biochemical parameters

The serum glucose (mg/dl) and serum cholesterol were significantly higher in control group in comparison to treatment groups T_2 and T_3 with subsequent increase in levels of fenugreek seeds @ 150 g and 250 g, respectively (Table 4 and 5). The levels of serum glucose concentration and serum cholesterol followed inversely proportional trend with amount of fenugreek seed supplementation. The low blood glucose concentration due to fenugreek supplementation might be due to the presence of an amino acid called 4-hydroxy isoleucine in fenugreek seeds which appears to act on pancreatic β-cells to increase production of insulin in the body. Higher amounts of insulin production may decrease the sugar that stays in the blood (Schryver, 2002). The findings of Raju *et al.* (2001) and Devi *et al.* (2003) that the ability of fenugreek seeds to modulate key glucose metabolizing enzymes such as hexokinase (glycolysis), glucose-6-phosphatase or fructose-1, 6-bisphosphatase (gluconeogenesis) might be considered as a possible mechanism. The average total protein, serum

Table 2. Average milk yield (kg) of experimental buffaloes at weekly intervals under different dietary treatments

Period (Week)	Treatments		
	T_1	T_2	T_3
Initial	6.91±0.66	8.21±0.74	6.70±1.00
I	9.15±0.52	10.98±0.66	9.41±0.83
II	10.91±0.65	11.03±0.76	10.75±0.60
III	11.60±0.67	11.07±0.60	11.81±0.51
IV	11.78±0.91	11.68±0.51	12.20±0.47
V	11.98±0.78	10.28±1.24	12.11±0.39
VI	11.38±1.10	10.85±0.87	13.00±0.41
VII	12.01±0.70^{ab}	11.06±0.51^{a}	13.48±0.52^{b}
VIII	11.80±0.99	11.10±0.31	12.75±0.78
IX	11.93±0.85	11.68±0.43	12.63±0.83
X	12.15±0.63	11.65±0.47	13.76±1.20
XI	12.33±0.68	12.06±0.46	13.11±0.38
XII	12.50±0.62	11.58±0.36	12.95±0.60
XIII	12.58±0.81	12.08±0.31	12.78±0.48
XIV	12.41±0.89	11.88±0.82	12.66±0.51
XV	12.36±0.88	11.83±0.57	12.31±0.60
XVI	11.98±0.57	11.53±0.47	12.26±0.41
XVII	11.38±1.05	10.96±0.54	11.60±0.68
XVIII	11.31±0.91	10.95±0.41	10.76±0.36
XIX	12.06±0.99	11.28±0.54	10.80±0.50
XX	10.38±1.62	11.11±0.43	10.98±0.30
XXI	9.46±1.51	11.33±0.52	10.50±0.45
XXII	10.51±1.44	11.41±0.72	9.68±0.40
XXIII	10.68±1.30	11.15±0.63	9.80±0.24
XXIV	10.13±1.09	10.00±0.46	9.90±0.32
XXV	9.88±0.94	9.66±0.24	9.41±0.20
XXVI	9.28±0.80	9.43±0.19	9.16±0.24
Overall mean	11.21±0.76	11.11±0.39	11.46±0.27

The mean values with different superscripts in a row differ significantly (P<0.05)

globulin, serum albumin, SGOT and SGPT values were non-significant among all the treatment groups.

CONCLUSION

The supplementation of Fenugreek (*Methi*) in lactating Murrah buffaloes @ 150 g and 250 g has no adverse effect on milk production as well as on milk composition. The supplementation of Fenugreek significantly affects the serum cholesterol levels in the lactating Murrah buffaloes.

Table 3. Average fat (%) in milk of experimental buffaloes at fortnightly intervals under different dietary treatments

Period (Fortnightly)	Treatments		
	T_1	T_2	T_3
Initial	8.10±0.02	8.08±0.01	8.14±0.07
I	7.05±0.20	7.17±0.04	7.08±0.23
I	6.96±0.14	7.15±0.04	7.08±0.26
III	6.55±0.12	6.90±0.05	6.85±0.15
IV	6.41±0.10	6.51±0.10	6.56±0.05
V	6.41±0.01	6.68±0.08	6.68±0.74
VI	6.43±0.01	6.50±0.10	6.68±0.08
VII	6.55±0.12	6.65±0.10	6.66±0.12
VIII	6.96±0.14	7.15±0.04	7.08±0.26
IX	6.96±0.04	7.08±0.26	7.15±0.04
X	7.05±0.20	7.17±0.04	7.08±0.26
XI	6.93±0.12	7.06±0.02	7.15±0.04
XII	7.15±0.04	7.36±0.08	7.27±0.12
XIII	7.55±0.16	7.60±0.17	7.70±0.12
Overall mean	6.93±0.07	7.07±0.05	7.09±0.05

The mean values with different superscripts in a row differ significantly (P<0.05)

Table 4. Average serum glucose (mg/dl) values of experimental buffaloes at monthly intervals under different dietary treatments

Period (Month)	Treatments		
	T_1	T_2	T_3
Initial	64.38±0.29	64.40±0.30	64.28±0.29
I	67.55^{c}±0.17	66.37^{b}±0.17	64.17^{a}±0.25
II	68.01^{c}±0.07	64.84^{b}±0.17	63.12^{a}±0.07
III	69.59^{b}±0.15	69.43^{b}±0.14	66.42^{a}±0.14
IV	68.42^{c}±0.11	67.01^{b}±0.04	64.46^{a}±0.13
V	69.38^{c}±0.14	67.24^{b}±0.08	65.02^{a}±0.03
VI	69.31^{c}±0.08	68.38^{b}±0.11	65.25^{a}±0.11
Overall mean	67.92^{c}±0.03	66.97^{b}±0.03	64.67^{a}±0.05

The mean values with different superscripts in a row differ significantly (P<0.05)

Table 5. Average serum cholesterol ((mg/dl)) values of experimental buffaloes at monthly intervals under different dietary treatments

Period (Month)	Treatments		
	T_1	T_2	T_3
Initial	88.46±0.38	87.85±0.45	88.83±0.53
I	99.8±0.70	99.23±0.28	98.62±0.18
II	106.97^{b}±0.78	101.95^{a}±0.73	100.82^{a}±0.43
III	104.16^{b}±0.93	102.73^{b}±0.55	100.21^{a}±0.69
IV	104.98^{c}±0.53	98.48^{b}±0.23	94.89^{a}±0.69
V	101.67^{b}±0.55	88.86^{a}±0.99	87.42^{a}±0.53
VI	98.48^{b}±0.23	87.01^{a}±0.46	87.64^{a}±0.53
Overall mean	100.65^{c}±0.20	95.16^{b}±0.27	94.06^{a}±0.10

The mean values with different superscripts in a row differ significantly (P<0.05)

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Haryana Vet. (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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Received: 17.03.2022; Accepted: 26.05.2022

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

How to cite: Unni, V., Abishad, P., Juliet, S., John, L., Nambiar, P., Latha, C., Vinod, V.K., Asha, K., Vergis, J., Kurkure, N.V., Barbuddhe, S.B. and Rawool, D.B. (2023). *In vitro* antimicrobial activity of piperine against multi-drug resistant *Salmonella* spp.. *Haryana Vet.* **62(1)**: 5-7.

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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