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Antibacterial Activities of Trans-cinnamaldehyde against *Salmonella enterica* Isolated from Calf diarrhea

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ABSTRACT

Background and aims: Infectious diarrhea with Salmonella spp. is the most significant cause of morbidity and mortality in neonatal calves that need emergency treatment. Trans-cinnamaldehyde (TC) is among the secondary metabolites of cinnamon trees that can be used as antimicrobial agents. The aim of the present study was to evaluate antibacterial activity of Trans-cinnamaldehyde on *Salmonella enterica* isolated from calf diarrhea.

Methods: 150 stool specimens were collected from the calves refereed to veterinary hospital and clinic of Urmia University during the period of winter to spring, 2018. Then, *Salmonella enterica* was isolated by culture and confirmed by molecular method. Antimicrobial activity was determined by agar disk diffusion method.

Results: The results of disk diffusion test showed that the Trans-cinnamaldehyde has an antibacterial effect against *Salmonella enterica*, with a diameter of the inhibition zone of 23 mm, which was very effective compared with the diameter of the inhibition zone caused by standard antimicrobial agents such as gentamicin and chloramphenicol that were 13.1 and 14 mm in the inhibition zone, respectively.

Conclusion: The results of this study indicate that cinnamaldehyde has antibacterial properties and can be used as a cheap, safe, and available source for therapeutic use in *Salmonella enterica*-induced bacterial infections.

Keywords: Essential Oil, Trans-cinnamaldehyde, Salmonella enteric, Calve diarrhea.

Original article

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INTRODUCTION

Calf diarrhea is the most important disease and a major cause of economic loss to cattle producers and livestock production industry [1]. According to the National Animal Health Monitoring System (NAHMS) for the U.S., 57% of unweaned calf mortality was due to diarrhea especially in calves less than one month old [2]. Calf diarrhea is attributed to both infectious and non-infectious factors [1]. Diarrhea induced by infectious organisms is the most significant cause of morbidity and mortality in neonatal ruminants throughout the world and can be caused by many pathogens including viruses, protozoa, and bacteria [3]. Among bacteria, *Salmonella* spp. is the most common bacterial etiologic agent of calf diarrhea during the first weeks of life [3]. It is economically the most important pathogen [4], although other bacteria have also been identified as causes of enteric diseases. Diarrhea due to *Salmonella* infection is watery and mucoid with the presence of blood and fibrin [5]. The lesions frequently observed in affected calves involve the pseudomembrane on the mucosa of the small intestine as well as enlargement of the mesenteric lymph nodes and also cause subclinical infections, latent infections, and acute, fatal septicemia and abortion [6]. Human epidemiological and medical studies suggest that this organism also contributes to the etiology of human diseases. Infected cattle can serve as a source of zoonosis through foodborne routes or direct contact [1]. *Salmonella* is a gram-negative bacterium that has several actinicity factors that increase its pathogenicity. One of these factors is the presence of flagella. This bacterium has two types of flagella that make connection with small intestinal epithelial cells. The genus of *Salmonella* has 3 known species, including *Salmonella chlora* (with one serotype), *Salmonella typhi* (with one serotype), and *Salmonella enteritidis* (with about 2000 serotypes). *Salmonella enterica* is the main cause of salmonellosis in most livestock species [7]. Currently, there is some drug approved for the treatment of salmonellosis in animals. Sulfonamides and gentamicin are used to treat salmonella-induced diarrhea in ruminants' neonates. These drugs have a number of side effects, including liver and kidney failure with toxic nephrosis and hemorrhage, as well as removing the natural micro flora of the digestive tract [8]. Nowadays, due to the creation of microbial resistance to antibiotics, the use of other safe sources such as plants-derived agents and their compounds has been suggested as an alternative to replace synthetic antibiotics. Recently, the results of many studies show that some plants have the ability to inhibit the growth of microorganisms [9]. Trans-cinnamaldehyde (TC) is an aromatic aldehyde that is extracted as a major component of the bark extract of cinnamon Cinnamomumzeylandicum). This structure has been reported to possess antimicrobial activity against a wide range of foodborne pathogens [10]. Cinnamaldehydeis is a compound of interest that can be used for development as food antimicrobial agents due to their demonstrated activity against both gram-positive and gram-negative bacteria, including organisms that are of concern for safety [10]. Therefore, the aim of the present study was to evaluate antibacterial activity of Trans-cinnamaldehyde on *Salmonella enterica* as a pathogen that is isolated clinically from calf sufferring diarrhea.

METHODS

In this study, 150 stool specimens were collected from diarrheic calves referred to veterinary hospital and clinic of Urmia University during the six-month period in winter and spring, 2018. These samples were immediately transported under cold-chain conditions and within the shortest time possible to the microbiology laboratory of the Faculty of Veterinary Medicine, which were then suspended in centrifuge, applied to the cysteine fluid celandine, and finally incubated at 37° C for 24 hours. In order to reach the pure colonies of *Salmonella*, the contents of the enrichment medium were sufficiently cultured in a selective medium of Salmonella-Shigella (SS agar) and then kept at 37° C for 24 hours. After this period, colonies of *Salmonella* under sterile conditions were harvested and tested by standard biochemical and biological tests such as transferring to urea, Simon citrate agar, SIM, lysine decarboxylase, and MRVP to identify and validate the phenotypes of the isolates. Finally, the strain was identified by the use of biochemical profiles according to the recommendations of the manual of clinical microbiology. Isolated bacteria with negative lactose biochemical characteristics, positive H_2S, negative indol, positive red-methyl, and venipuncture, positive citrate, negative urease, and positive lysine decarboxylase were considered as isolates belonging to the genus *Salmonella* [11,12,15]. It should be noted that all the media used in this study were made by Merck-Germany.

Disk Agar Diffusion

The International Institute for Laboratory Standard (CLSI) guidelines was used to conduct the test. Bacterial suspensions equivalent to 0.5 McFarland turbidity were prepared in sterile normal saline solution from clinical and reference isolates. A sterile swab was dipped into the inoculum tube containing bacterial suspensions and then was cultured on the Müller-Hinton agar (Merck®, Germany). Sterile filter paper discs (6 mm in diameter) were impregnated with Trans-cinnamaldehyde (Sigma®, USA) (15 µL) for 10–15 minutes, allowed to dry completely for 20–25 minutes, and then evenly placed on the surface of previously inoculated cultures. Gentamicins (10µg), chloramphenicol (30µg) antibiotic discs (Merck®, Germany) were positive controls and sterile diluent (0.1% peptone water) was negative control for comparison of inhibition zone with sample. Plates were incubated at 37°C for 24 hours, until visible growth of bacteria was evident in control plates. Clearly, visible inhibition zones around discs were measured in 3 directions and averaged. The antibacterial activity was expressed according to the diameter of inhibition zone produced by extract against test bacteria [13, 14, 15].

RESULTS

Out of 150 fecal samples collected from diarrheic calves, 142 (94.6%) bacterial isolates were recovered, from which *Salmonella* serovars were used for this study. The serotyping of *Salmonella* isolates shows *Salmonella enterica* (76.6%) as a most commonly detected isolate.

Results of in-vitro sensitivity tests

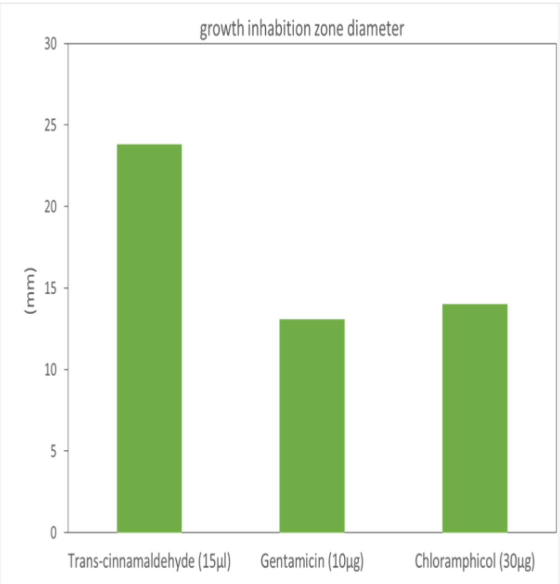

Fig 1. Comparison of antibacterial Activities of trans-cinnamaldehyde against *Salmonella enterica*

of *Salmonella* isolates against 2 standard antimicrobial agents revealed that *Salmonella enterica* isolates showed high sensitivities against chloramphenicol. The results of disk diffusion test showed that the Trans-cinnamaldehyde has very high antibacterial activity against *Salmonella enteric*. Trans-cinnamaldehyde reveled inhibition zone with a diameter of 23 mm, while the diameters of the inhibition zone caused by gentamicin and chloramphenicol were 13.1 and 14 mm, respectively. A significant difference ($p<0/05$) of cinnamaldehyde was observed (Figure 1).

DISUSSION

Calf diarrhea in neonatal period remains as one of the most important problems faced by livestock, leading to great economic losses. Commonly, calves are at greatest risk of diarrhea within the first month of life although the incidence of diarrhea decreases with age [16]. Enteropathogens have a high level of resistance to commonly used antibiotics and resistance to new drugs emerges very fast. Diarrheal diseases are far more common among calves and the search for new drugs with antibacterial activity against enteropathogens has a public health priority [1, 2]. New agents derived from medicinal plants result in recovery without side effects. This is very important, since a major problem with antibiotics is a common failure to accomplish treatment, which in turn favours selection of resistant bacterial strains. Previously, the world was once again aware of the side effects and losses of many chemical drugs. With the development of research on the unwanted effects of chemical drugs, the use of herbal medicines has been re-considered [17]. Most essential oils of the plants have antimicrobial activity, which is mainly related to their phenolic compounds. The higher the amount of phenolic material of the essential oil, the more antimicrobial activity is [18, 19]. The ethnobotanical studies showed a previously reported antimicrobial activity of Cinnamomumzeylandicum. Antibacterial activities of essential oils of cinnamon and Trans-cinnamaldehyde against *Staphylococcus* spp. isolated from clinical mastitis of cattle and goats were demonstrated [20]. Other research showed that Trans-cinnamaldehyde has a bacteriostatic and bactericidal activity against the *Staphylococcus* spp [20]. Evaluation of antibacterial effect of Trans-Cinnamaldehyde on *salmonella enteritidis* and *campylobacter jejuni* in chicken drinking water indicates that TC is very effective in killing *S. enteritidis* and *C. jejuni* organisms [10]. In another study, antibacterial activity of Cinnamaldehyde and Sporan against *Escherichia Coli* and *Salmonella* were evaluated and results showed Cinnamaldehyde was highly effective against both *E. coli* O157:H7 and *Salmonella* spp. [21, 25]. Synergetic effect was reported for trans-cinnamaldehyde, as low concentrations of trans-cinnamaldehyde elevate the antimicrobial action of clindamycin, suggesting a possible clinical benefit for utilizing these natural products for combination therapy against *Candida*. *difficile* [22]. The results of this study showed that the Trans-cinnamaldehyde has an antibacterial effect against *Salmonella enterica*, and its inhibitory effect was greater than that of gentamicin and chloramphenicol. According to the results of our research and previous research, trans-cinnamaldehyde has an antimicrobial effect that is made by binding the carbonyl group of proteins of bacteria and preventing the decarboxylation of amino acids [23].

Studies are needed to characterize the activities mechanisms of Trans-cinnamaldehyde and to study its toxicity to further define the potential therapeutic benefit of it or the risk that accompanies its oral administration.

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Studies are needed to characterize the activities mechanisms of Trans-cinnamaldehyde and to study its toxicity to further define the potential therapeutic benefit of it or the risk that accompanies its oral administration.

CONCLUSION

The results of this study indicate that cinnamaldehyde has antibacterial properties and can be used as a cheap and available source for therapeutic use in some Salmonella-induced infections. In particular, cinnamaldehyde can be considered as a suitable substitute for synthetic antibiotics to treat the clinical Salmonellosis. Of course, more studies are needed in order to study the antibacterial effects of cinnamaldehyde and its compounds more accurately. Also, with regard to the potent antibacterial properties of this essential oil component, it seems that it could be used in veterinary medicine. Therefore, taking into account the detrimental effects of antibiotics, the use of medicine-based drugs can be a new horizon for controlling the diarrhea pathogens especially bacteria.

CONFLICT OF INTEREST

All authors disclose any finance and declare that there are not any potential conflicts of interest.

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