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Effect of Clove and Garlic oils on Minced Meat Quality and Some Foodborne Pathogens

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Abstract

Minced beef has very high nutritional and economic value. Minced meat is highly susceptible to microbial invasion with food poisoning risk. Clove and garlic oil extract enhance food's sensory properties and shelf life. In the current study, a total of seventeen samples of minced meat weighing 100 g for each were inoculated with *Staphylococcus aureus* and *Escherichia coli* to demonstrate the effect of clove and garlic oil extract on their growth. Each sample was divided into 8 parts (one part as a control, 3 parts for each clove and garlic oil extract (0.3, 0.5, and 1%) separately and one part for both clove and garlic oil extract mixture; 100g each). The results showed that the sensory properties of the examined minced meat samples treated with oil extract of clove and garlic were improved during cold storage (4± 1°C) by increasing the oil concentrations compared to the untreated control samples. Clove and garlic oil at different concentrations (0.3, 0.5 and 1%) have an inhibitory effect on *S. aureus* and *E. coli* growth. Clove has more inhibitory effect on *S. aureus* growth. Garlic oil was more effective on inhibiting the growth of *E. coli* than *S. aureus*. The mixture of two oils at 0.3% has a synergistic effect and improved their antibacterial effect using each alone at the same concentration. It was observed that adding essential oils (EOs) useful in maintaining minced meat quality and extending its shelf life.

Keywords: Minced meat; Garlic; Clove; *Staphylococcus aureus*; *Escherichia coli*

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1. Introduction

Meat products are gaining popularity because they represent quick, easily prepared meals with low prices from one side and render the processors to convert the various types of meat into unified products. Particularly, minced meat is habitually made from lean meat and used for different cooking and meals. **(Ahmed-Awatif and Sabiel, 2016)**. On the other side, meat products harbor different types of microorganisms along the chain through handling, processing, distribution, preparation, and storage (**Hassanien, 2004)**. The presence of *Staphylococcus aureus* in meat products may be attributed to direct contact with lesions in hands of workers or by sneezing and coughing. Food handlers are the common source of food contamination related with Staphylococcal outbreaks **(Jennifer Hait, 2012).**

Escherichia coli is an indicator of fecal contamination of food **(Synge, 2000)**. *E. coli* has been associated with food poisoning associated with contaminated meat products. The symptoms of *E. coli* food poisoning include bacteremia, pneumonia, neonatal meningitis, endovascular infections, urinary tract infections, deep wound infections, septicemia, and vertebral osteomyelitis **(Datta et al., 2012).**

Food additives enhance the appearance and shelf life of food. Food additives might be naturally extracted as herbs and spices or artificially prepared as calcium acetate and calcium oxide **(MFDS, 2013)**. Artificially prepared food additives could have potential toxicity, so consumers prefer natural food additives over synthetic ones **(Saleh et al., 2020)**. Therefore, the extraction of essential oils from plants is widely applied in the food industry. Essential oils possess antimicrobial and antioxidative properties, so their use to extend the shelf-life of food products could be very useful **(Juana Fernández-López and Manuel Viuda-Martos., 2018).**

Clove (*Syzigium aromaticum* L.) and garlic (*Allium sativum*) essential oil have antibacterial, antiviral, antifungal and anti-protozoal valuable effects on the cardiovascular and immune system **(Harris et al., 2001)**. They can inhibit the growth of *S. aureus* in meat products. Clove antibacterial activity is attributed to eugenol with humulene and caryophyllene **(Ibrahim-Hemmat et al., 2016)**. Garlic is one of the most used ingredients for flavor enhancement of food. The current study was planned to determine the effect of clove and garlic oils on improving the quality of minced meat.

So, the present study evaluated the efficacy of clove and garlic essential oils with different concentrations as natural preservatives against *E. coli* and *S. aureus* inoculated experimentally in minced meat. Clove and garlic essential oils improved the shelf life and presentation of meat products to introduce safe food for the consumer

2. Material and Method

2.1. Extraction of clove and garlic essential oils

The dried plant parts of clove *(Syzigium aromaticum* L.) and garlic (*Allium sativum*) were purchased from a local market (El Beheira governorate, Egypt). The dried clove buds were ground into a fine powder; the garlic seeds were subjected to hydro distillation (steam distillation; plant material in boiling water) using a Clevenger-type apparatus for 2 hours; The essential oils were extracted by steam distillation using a vertical steam distillation unit; the 2 essential oils extract at different concentration 0.3, 0.5, and 1%were prepared and stored at 4 °C in dark brown glass bottles until used.

2.2. Preparation of minced meat samples

A total of 17 imported minced beef samples weighing 100grams per each were purchased from local supermarkets in Damanhour, El Beheira governorate, Egypt. The samples were taken and transferred directly to the food hygiene laboratory in Animal Health Research Institute, Damanhur branch, using an ice box under complete aseptic conditions without undue delay.

Each sample was divided into 8 parts (one part as a control, 3 parts for each oil (0.3, 0.5, and 1%) and one part for mixed oils; 100g each). The samples were backed in polyethylene bags till inoculation with microorganisms.

2.3. Preparation of bacterial strains

Reference strains of coagulase-positive *S. aureus* (ATCC 25923) and *E. coli* O157 H7 (ATCC 35218) were obtained from the microbial strain bank of Animal Health and Research Institute Dokki, Giza, Egypt. Four to five isolated colonies of each tested strain were picked and inoculated into 5 ml sterile peptone water (0.1%, Merck, Germany) and incubated at 37^0C/24 h. From this culture, serial dilutions up to 10^{10} were platted on baired parker agar and eosin methylene blue agar (Merck, Germany) for *S. aureus* and *E. coli*, respectively, to determine the cell concentration. The cell count was adjusted to 10^5cfu/ml for *S. aureus* and *E. coli*, with tube dilution methods, as this is considered an infective dose to be inoculated into minced beef samples. The effective dose of enterotoxins may be achieved when the population of *S.* aureus reaches a level of > 10^5 cfu/g **(Stewart *et al.*, 2003)**.

2.4. Inoculation of minced meat with tested bacteria

The tested strains were inoculated onto minced meat (to obtain the required initial count >10^5 in minced meat, 10 ml of the 10^8 dilutions were injected into 100 g of minced meat) by pouring and swabbing over the meat surfaces and then thoroughly mixed by gentle squeezing of the sample bag by hands. Subsequently, the inoculated meat samples were kept for 20 minutes to allow attachment between the microorganism and minced meat and complete absorption of the inoculated bacteria; then bacteriological examination to confirm the infective dose of *S. aureus* and *E. coli*. The addition of two tested essential oils with different concentrations of 0.3, 0.5, and 1% to examined minced meat samples and thoroughly mixed **(Dubal et al., 2004)**.

The control and treated minced meat samples were packed in polyethylene bags, then labelled, identified, and stored at $4C^0$ in the refrigerator. All parts (control and treated) were subjected to microbiological examination at day zero (after 3 hours of treatment) and then periodically every day (24hrs) during storage for 5 days; trials were performed in triplicate. Furthermore, sensory evaluation was applied for the control and treated samples.

2.5. Sensory evaluation

Minced meat samples were exposed to sensory evaluation using semi-trained panellists. The panellists explained the experiment's nature without disclosing the samples' identities. The sensory evaluation was performed at room temperature using white light. The panelists were asked to mention their color, odor, texture, and overall acceptability preferences. The panellists gave a score for each item using the 9-point descriptive scale (Excellent, 9; Very very good, 8; Very good, 7; Good, 6; Medium, 5; Fair, 4; Poor, 3; Very poor, 2; Very very poor, 1.) was used to evaluate the overall acceptability **(Pearson and Tauber 1984)**.

2.6. Bacteriological analysis for S. aureus and E. coli

10 g of each prepared minced meat samples (control and treated) were homogenized with 90 ml of buffered peptone water, and ten-fold serial dilution was prepared; 0.1ml was spread on the dried surface of triplicate plates of baired parker agar for *S. aureus* count and EMB agar for *E. coli* count. Baired parker agar plates were incubated at 37ºC for 48 hours. Black shiny colonies with narrow white margins and surrounded by a clear halo zone were enumerated for *S. aureus*. While *E. coli* count was determined on EMB after incubation at 37 ºC for 24 - 48 hours, and metallic colonies were enumerated (**FDA 2001**). Reduction percentage was calculated according to the following:

Reduction% = ((Initial load – new count)/ Initial load) x 100

2.7. Statistical analysis

The data were analyzed using one-way variance analysis (ANOVA) with Duncan by SPSS® version 16.0. A statistical probability (*p*-value) less than 0.05 indicated a statistically significant difference between examined parts.

3. Results and Discussion

Natural food additives are required to enhance the quality of meat products without adverse effects **(Simitzis et al., 2008)**. The antimicrobial effects of essential oils (Eos) act as a natural method for food preservation techniques. Therefore, EOs are promising natural antimicrobial agents with prospective application in the food or pharmaceutical industries to control pathogenic microorganisms **(Rahman and Kang, 2009).**

In the current study, clove, and garlic oil extract (0.3, 0.5 and 1%) were used to explore their effect on minced meat quality. The results showed that the sensory properties of the examined minced beef samples treated with oil extract of clove and garlic during cold storage (4 ± 1 °C) were improved with increasing oil concentrations compared to the untreated (control) samples. Results in **(Tables 1** and **2)** showed that the samples treated with 1% garlic and clove oils each had the highest improvement of sensory qualities until the sixth day of cold storage, while samples treated with 0.3% of the same oils showed almost less improvement on the fourth or fifth day of the day at cold storage. It was also observed that the 0.3% of oil mixture showed better improvement in the samples inoculated with *S. aureus* until the fifth day of cold storage compared to the single oil. At the same time, it was less effective in the case of samples inoculated with *E. coli* until the third day of cold storage, when samples began to show signs of deterioration.

Our results were nearly agreed with **Ibrahim,Hemmat et al.,** (**2016)** and **Salem,Amany et al.,** (**2017),** who reported that essential oils (EOs) enhanced the sensory properties of minced beef samples, and more improvement occurs in samples inoculated with *S. aureus*. However, the high concentration of the oils enhances the organoleptic properties of the examined minced beef samples, especially the color and visual acceptance. It delays the signs of deterioration, but if clove oil is added at a high concentration, 1% alters the smell of the minced meat samples, which consumers may not accept. These results agree with **Gutierrez et al., (2009)** and **Soković et al.,** (**2010).**

As noted from the results obtained above in **Table 3**, clove oil at different concentrations (0.3, 0.5, and 1%) inhibited the *S. aureus* growth with different degrees of inhibition compared with the control sample at the same storage condition. The reduction percentages of clove oil at 0.3, 0.5, and 1% on *S. aureus* growth were 30.31, 100, and 100%, respectively, on the 3^{rd} day of the experiment; the highest reduction percentages occurred with the highest concentration of 1% at the 4^{th} of experiment which was 100% (complete inhibitory effect on the growth of *S. aureus*) which means that no microorganisms could be detected in the minced meat sample treated with clove 1% at 2^{nd}, $3^{rd}$and 4^{th}day of cold storage period at 4 ±1 ^{0}C. However, our finding explained that clove possesses an antibacterial effect against *S. aureus* with different concentrations and a high reduction percentage occurred with higher concentrations of 1%, 0.5%, and 0.3%. Thus, increasing the shelf life of minced beef samples for more than 4-5 days of the storage period, but it was noted that with high concentration, a powerful odor of clove was detected, some panelists and consumers might not accept that. Our results are compatible with **Ibrahim–Hemmat et al.,** (**2016)**, who explained that complete inhibition of *S. aureus* occurred on the 4^{th} day of the experiment with clove 1%.

Also, the results in **Table 3** showed that garlic oil was effective in inhibiting the growth of *S. aureus* at different concentrations (0.3, 0.5 and 1%) with different degrees of inhibition compared with the control sample at the same storage condition. The highest reduction ratio of garlic oil at 0.3, 0.5, and 1% was 32.05, 100 and 100%, respectively, on different days of storage. On the experiment's 2^{nd}, 3^{rd}, and 4^{th} days, the highest reduction percentages (complete inhibitory effect on *S. aureus* growth) accompanied with the concentration of 0.5 and 1% of garlic oil. However, our results indicated that garlic possesses an antibacterial effect against *S. aureus* at different concentrations and that the high reduction percentages obtained at higher concentrations of 1%, 0.5%, and 0.3%, thus increasing the shelf life of minced meat samples for more than 4-5 days of storage period. Still, it was noted that with the higher concentration, a strong odor of garlic was detected that some panellists and consumers might not accept.

For *E. coli* growth, **Table 4** explained that clove oil effectively inhibited *E. coli* growth at different concentrations (0.3, 0.5, and 1%) with various degrees of inhibition concerning the control part at the same storage condition. The highest reduction percentage of *E. coli* growth obtained with clove oil at different concentrations (0.3, 0.5, and 1%) were 30.12, 100, and 100% on the 1st day of the experiment, respectively. The highest reduction percentage occurred with 0.5% on the 3rd day of the storage period, which was 100% (complete inhibitory effect on *E. coli* growth), which mean microorganism can't be detected in examined minced meat sample treated with clove 0.5% at 3rd day of cold storage period at 4 ±1 ^{0}C. At the same time, it was observed that the count of *E. coli* began to increase from the 3rd day of the experiment in the treated minced meat sample with clove 0.3% but still lower than the control sample, which means clove 0.3% less effective than higher concentration.

Also, **Table 4** revealed that garlic oil was effective in the growth inhibition of *E. coli* with different concentrations (0.3, 0.5, and 1%) with various degrees of inhibition concerning the control part at the same storage condition. The highest reduction percentages of garlic (0.3, 0.5, and 1%) were 31.43, 100, and 100% on the 1st day of the experiment, respectively. The highest reduction percentage occurred with the highest concentration of 0.5% on the 2nd day of the storage period, which was 100% (complete inhibitory effect on *E. coli*), which mean microorganism can't be detected in examined minced meat sample treated with clove 0.5% at 2nd day of cold storage period at 4 ±1 ^{0}C. In contrast, it was reported that the number of *E. coli* began to increase from the third day of the experiment in the minced meat sample treated with garlic at 0.3% but still less than in the control sample, which means that garlic is 0.3% less effective than the higher concentration.

The discussed results in **Table 5** showed that clove oil has a strong antibacterial effect on *S. aureus* than *E. coli. Still,* garlic oil of different concentrations (0.3, 0.5, and 1%) has a slightly higher effect on growth inhibition of *E. coli* than *S. aureus.* This agreed with **Gupta -Charu et al., (2008),** who revealed that clove oil had good antibacterial activity against all gram-positive and gram-negative bacteria. Clove oil appears to have a potential effect for use as a preservative. Other similar results were reported by **Abdel Monein-Sulieman et al., (2007)** and **Babu-Jagadeesh et al., (2011),** who proved that EO of clove has potential antimicrobial activity against *S. aureus* and *E. coli.*

Tables 3 and **4** showed that the mixture of the two oils at 0.3% was better in growth inhibition of *S. aureus* and *E. coli*, with a higher reduction percentage than 0.3% concentration for each. That means that the mixture of oils of 0.3% has a synergistic effect and improves the antibacterial effect of garlic at 0.3% and clove at 0.3% rather than the oil alone at this concentration. In addition, the oil mixture possesses an accepted and preferable odour and flavor to overcome the unaccepted flavor of high concentration, particularly clove oil. The results of the mixed oil extract synergistic effect agree with **Das et al.,** (**2003)** and **Kota and Paladi, (2013)** who stated that the synergistic effect is generally a result of components of one spice aiding the other and improving the total efficacy.

The antibacterial activity of cloves is certified in eugenol content (70-90%) (2-methoxy-4-allyl phenol) with humulin and caryophyllene and has a high content of tannin (10-19%) that provides the additional antimicrobial activity. In addition, clove oil's antimicrobial effect is associated with the inactivation of microbial enzymes, adhesion, and cell envelope proteins **(De Guzman, 1999; Marin et al., 2004).**

Garlic oil contains allicin, the most potent antibacterial agent in crushed garlic extracts. The oil rich in organic sulfur compounds inhibited many growing bacteria such as E. coli & S. aureus, subsequently increasing the shelf life and enhancing the organoleptic characteristics of meat **(Hahn, 1996; Jolly and Menon, 2015).**

Table 1. Physical characters of untreated and treated meat inoculated with *Staphylococcus aureus*

Sample	**3 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	8	6	4	2	0
Garlic 0.3%	8	7	6	5	4
Garlic 0.5%	9	8	8	7	7
Garlic 1%	9	8	8	7	7
Clove 0.3%	8	8	6	6	4
Clove 0.5%	8	8	7	6	6
Clove 1 %	9	8	8	8	7
Mix of the 2 oils 0.3%	9	8	7	6	5

Table 2. Physical characters of untreated and treated meat inoculated with *Escherichia coli*

	3 hours	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	8	7	5	3	2
Garlic 0.3%	9	8	7	6	4
Garlic 0.5%	9	8	7	6	5
Garlic 1%	9	8	8	8	7
Clove 0.3%	9	8	7	5	3
Clove 0.5%	9	8	8	7	6
Clove 1 %	9	8	8	7	7
Mix of the 2 oils 0.3%	9	8	6	4	3

Table 3. Effect of clove and garlic essential oils on *Staphylococcus aureus* count (log cfu /g) and its reduction% experimentally inoculated into minced meat samples and stored at 4± 1º C.

Treatment		**3 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	Mean±SD	6.04±0.11^{a}	5.95±0.83^{a}	6.09±0.91^{a}	6.15±1.33^{a}	6.99±1.31^{a}
Clove 0.3%	Mean±SD	5.52±0.76^{b}	4.93±0.95^{c}	4.47±1.26^{b}	4.19±0.99^{b}	5.31±1.03^{b}
	Reduction %	8.63	17.19	26.52	30.31	24.08
Clove 0.5%	Mean±SD	5.00±1.18^{c}	3.91±1.23^{d}	3.46±0.77^{c}	0.00±0.00^{c}	0.00±0.00^{d}
	Reduction %	17.22	34.26	43.14	100.00	100.00
Clove 1%	Mean±SD	3.97±1.23^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}
	Reduction %	34.31	100.00	100.00	100.00	100.00
Garlic 0.3%	.Mean±SD	5.88±0.91^{b}	5.22±1.13^{b}	4.36±1.32^{b}	4.43±1.18^{b}	4.75±1.05^{c}
	Reduction %	2.67	12.26	28.35	26.41	32.05
Garlic 0.5%	Mean±SD	5.30±0.70^{b}	4.52±1.30^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}
	Reduction %	12.32	24.10	100.00	100.00	100.00
Garlic 1%	Mean±SD	4.66±0.71^{d}	3.88±0.61^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}
	Reduction %	22.84	34.80	100.00	100.00	100.00
Clove+ Garlic 0.3%	Mean±SD	5.30±1.00^{b}	3.63±1.23^{d}	3.56±1.23^{c}	4.07±0.88^{b}	4.34±0.99^{c}
	Reduction %	12.23	38.91	41.49	32.39	37.85

Each reported value is the mean ± SD.
Means within the same column followed by different lower-case letters are significantly different ($p < 0.05$).

Table 4. Effect of clove and garlic essential oils on *Escherichia coli* count (log cfu /g) and its reduction % experimentally inoculated into minced meat samples and stored at 4± 1º C.

Treatment		**3 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	Mean±SD	5.24±0.27^{a}	5.47±0.47^{a}	5.53±0.44^{a}	5.67±0.49^{a}	5.97±0.76^{a}
Clove 0.3%	Mean±SD	5.08±0.47^{b}	4.43±0.51^{b}	3.87±0.54^{b}	4.63±0.50^{b}	4.86±0.61^{b}
	Reduction %	3.11	19.14	30.12	18.24	18.60
Clove 0.5%	Mean±SD	4.39±0.65^{d}	3.89±0.59^{c}	3.26±0.76^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Reduction %	16.25	28.88	41.09	100.00	100.00
Clove 1%	Mean±SD	4.36±0.71^{d}	3.73±0.59^{c}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}
	Reduction %	16.86	31.85	100.00	100.00	100.00
Garlic 0.3%	Mean±SD	4.67±0.60^{c}	4.37±0.51^{b}	3.79±0.57^{b}	3.93±1.00^{c}	4.58±0.70^{bc}
	Reduction %	10.90	20.12	31.43	30.72	23.25
Garlic 0.5%	Mean±SD	4.46±0.55^{d}	3.87±0.43^{c}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}
	Reduction %	14.95	29.23	100.00	100.00	100.00
Garlic 1%	Mean±SD	4.36±0.61^{d}	3.55±0.46^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}
	Reduction %	16.86	35.25	100.00	100.00	100.00
Clove+ Garlic 0.3%	Mean±SD	4.63±0.42^{c}	3.83±0.45^{c}	3.64±0.49^{b}	3.84±0.53^{c}	4.20±0.57^{c}
	Reduction %	11.81	30.12	34.18	32.29	29.73

Each reported value is the mean ± SD.
Means within the same column followed by different lower-case letters are significantly different ($p < 0.05$).

Table 5. Comparison between the effect of clove and garlic essential oil on *Staphylococcus aureus* and *Escherichia coli* reduction % in chilled minced meat at 4 ± 1º C.

Treatment		**3 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control (Log cfu/ g)	*Staph.aureus* count log cfu/ g	6,04±0.11^{a}	5,95±0.83^{a}	6.02±0.91^{a}	6,9±1.33^{a}	6,99±1.31^{a}
	E. coli count	5,24±0.27^{a}	5,47±0.47^{a}	5.53±0.44^{a}	5.67±0.49^{a}	5.97±0.76^{a}
Clove 0.3%	*Staph.aureus* (R)%	8.63	17.19	26.52	30.31	24.08
	E. coli (R)%	3.11	19.14	30.12	18.24	18.60
Clove 0.5%	*Staph.aureus*(R)%	17.22	34.26	43.14	100	100
	E. coli (R)%	16.25	28,88	41.09	100	100
Clove 1%	*Staph.aureus* (R)%	34.31	100	100	100	100
	E. coli (R)%	16.86	31.85	100	100	100
Garlic 0.3%	*Staph.aureus* (R)%	2.67	12.26	28.35	26.41	32.05
	E. coli (R)%	10.90	20.12	31.43	30.72	23.25
Garlic 0.5%	*Staph.aureus* (R)%	12.32	24.10	100	100	100
	E. coli (R)%	14.25	29.23	100	100	100
Garlic 1%	*Staph.aureus* (R)%	22.84	34.80	100	100	100
	E. coli (R)%	16.86	35.25	100	100	100
Clove+ Garlic 0.3%	*Staph.aureus* (R)%	12.23	38.91	41.49	32.39	37.85
	E. coli (R)%	11.81	30.12	34.18	32.29	29.73

Each reported value is the mean ± SD.
Means within the same column followed by different lower-case letters are significantly different ($p < 0.05$).

4. Conclusion

This study has shown that all different concentrations of garlic and clove essential oils have antimicrobial properties on growth inhibition of *S. aureus* and *E. coli* inoculated in minced meat. Clove showed the maximum antibacterial effect on growth inhibition of *S. aureus*, especially with a concentration of 1%, enhancing the organoleptic aspects of minced beef compared to control samples with its accepted odor followed by garlic oil. Garlic oil was more effective in the growth inhibition of *E. coli* than clove oil. The mixture of both oils at 0.3% was more effective than each oil alone at the same concentration and possessed a synergistic effect. It was observed that using garlic and clove could be useful in maintaining the quality of meat and meat products, extending shelf life, preventing economic losses, and providing the consumer with foods containing natural additives that appear to be healthier than meats of industrial origin.

Conflict of interest: There are no conflicts of interest stated by the authors.

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