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ORIGINAL ARTICLE

Anticoccidial effects of tannin-based herbal formulation (*Artemisia annua*, *Quercus infectoria*, and *Allium sativum*) against coccidiosis in broilers

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Abstract
Background Avian coccidiosis is considered among the infectious disease of high cost in the poultry industry. Herbal extracts are safe and reliable substitute anticoccidial drugs for chemical feed additives as they do not sequel to drug resistance and tissue remnants.
Objective The current study aimed to assess the anticoccidial effect of an herbal complex of 3 plants (*Artemisia annua*, *Quercus infectoria*, and *Allium sativum*) in broiler chickens compared to toltrazuril anticoccidial.
Methods This experiment used one hundred twenty broiler chickens and divided them into four equally numbered groups. All the groups, except group (D), were experimentally infected with mixed *Eimeria* spp. (*E. tenella*, *E. maxima*, *E. necatrix* and *E. brunetti*) on day 14. Group (A) was treated with a herbal mixture, containing 75% *Quercus infectoria* with a minimum of 30% total tannin, 16% *Artemisia annua* with a minimum of 0.02% artemisinin, and 9% *Allium sativum* with a minimum of 0.4% total phenol contents. Group (B) was treated with toltrazuril. Group (C) did not have any treatment. Group (D) was healthy all the experiment period as a negative control group. During a 42-day breeding period, the study examined clinical signs, weight gains, feed conversion ratio, lesions scoring, casualties, and the number of oocysts in different bird groups.
Results Group (D) showed the most significant weight gain, indicating the economic damage caused by coccidiosis. The best feed conversion ratio was observed in the unchallenged group, and coccidiosis negatively affected it in other groups. Clinical signs of dysentery, diarrhea, and lethargy were seen post-challenge but improved with treatment. Group (D) showed no losses; others had casualties and coccidiosis lesions. Lesion scores were lowest in the group (D), and the herbal mixture improved performance. The herbal mixture and toltrazuril reduced oocyst counts in feces earlier than the untreated group.
Conclusion In conclusion, the anticoccidial activity of the mentioned herbal complex recommends its use as an alternative anticoccidial agent to chemotherapeutic drugs for controlling coccidiosis.

Keywords Chicken · Coccidiosis · Treatment · *Eimeria* · Herbs

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Introduction

Avian coccidiosis is a significant enteric disease in poultries that has the potential to impose a substantial economic consequence on the profitability of the farms (Blake et al. 2020). Its worldwide reputation comes from its notable impact on poultry industries, among parasitic diseases (Allen et al. 2005). Coccidiosis imposes a significant financial loss to the poultry enterprise, mainly due to healing or prophylactic medications and further because of the effect of the illness on the bird's well-being (Idris et al. 2017; Remmal et al. 2011). *Eimeria* species multiply inside the bird's intestinal tract, inflicting considerable tissue damage (Ghaniei et al. 2023). The tissue damage can impede digestion, feeding, and nutrient absorption (El-Hack et al. 2021). The outcome can be dehydration, poor skin pigmentation, blood loss, and proliferated vulnerability to other diseases, including necrotic enteritis, which can lead to death (Chapman 2009; Shirley and Millard 1986). The usage of chemotherapeutic agents is the primary established approach to controlling coccidia. Good results have been achieved in avian coccidiosis vaccines, especially in broiler breeders (Khater et al. 2020; Tewari and Maharana 2011). However, including multiple species of *Eimeria* in one vaccine can lead to notable derangements in weight gain and feed conversion, which is an unpleasant issue of using vaccines to control coccidiosis (Alfaro et al. 2007). Anticoccidial drug resistance and probable adverse effects on human health are the motivations to find more reliable substitutes for controlling coccidiosis (Nogueira et al. 2009). To develop new strategies for managing coccidiosis, it is vital to research extra interactions between *Eimeria* species and chickens with pertinent information about a bird's immune system (Muthamilselvan et al. 2016). So far, the poultry industry has excessively relied on anticoccidial drugs for treatment and prevention, while the drastic use of such medications has resulted in the extension of resistance (Abbas et al. 2011). Up to this point, although chemoprevention drugs and anticoccidial feed additives have managed to control coccidiosis, the challenge has been intricated due to the emergence of drug resistance and the toxic effects on animal health (Abbas et al. 2011). In addition, drug remnants in poultry meat and other products are a potential constraint to consumers (Peek and Landman 2011). Consequently, developed and underdeveloped countries seek alternative strategies for more efficient and reliable disease control (Sánchez-Hernández et al. 2019; Sundar et al. 2017). Among alternative approaches, herbal compounds are the potential candidates for managing avian coccidiosis (Bozkurt et al. 2012; Crespy and Williamson 2004). The mentioned approach is not a newly found concept. Phytogenic feed additives (often called botanicals or phytobiotics), such as extracts and essential oils of herbs and spices, have been investigated as potential sources of compounds with antimicrobial and anticoccidial activity (Nidaullah 2010; Youn and Noh 2001). Botanical components have displayed noticeable insecticidal, antioxygenic, anti-mycotic, anti-viral, and anti-parasitic properties, and these activities are perhaps connected to the function of these compounds in herbs (Elmahallawy et al. 2021; Yong et al. 2020). The use of herbal extracts and their supplements as feed additives has improved over the past few years due to their hypocholesterolemic, antibacterial, and anti-oxidation activity (Arafat and Abbas 2018). The allicin molecule which exists in *Allium sativum* (*A. sativum*) is an effective antioxidant that prevents lipid peroxidation, which leads to a hepatoprotective effect (Waqas et al. 2018). Furthermore, other compounds of *A. Sativum*, like Ajoene, DTS, and allyl methyl sulfide are responsible for the antibacterial, and antifungal (Bhatwalkar et al. 2021), antiprotozoal (Dorrigiv et al. 2020), and antiviral effects (Alagarsamy et al. 2018). *Quercus infectoria* (*Q. infectoria*) has medicinal effects such as antimicrobial, astringent, gastroprotective, anti-inflammatory, and antidiabetic. *Quercus infectoria*, containing tannins, gallic acid, gallotannins, and other compounds, controls eimeriosis in birds, like oocyst output and reduction of mortality and lesion score (Ardestani et al. 2019; Elham et al. 2021). *Artemisia* herb contains bioactive components and rich nutrients, most of which can be used as therapy; therefore, their extract or leaf powder can also be utilized as a natural herbal medicine feed additive (van der Kooy and Sullivan 2013). Additionally, the plant *Artemisia annua* (*A. annua*), with its antimalarial effects, has been analyzed for its diverse biologic effects (Feng et al. 2020). Artemisinin is one of the main potent components of *A. annua* (Shahrajabian et al. 2020).

In this study, a comparative model was designed to evaluate the effectiveness of the anticoccidial development of an herbal formulation based on three plants. The plants used in the herbal formulation included extracts of *Artemisia annua, Quercus infectoria,* and *Allium sativum*.

Materials and methods

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. (IR.UM.REC.1401.129)

Birds, housing, and grouping

On the whole, 120 one-day-old Ross 308 broiler chicks were bought from a local incubation center. The chicks were moved to the particular facility of the Faculty of the Veterinary Medicine of the Ferdowsi University of Mashhad and kept on a slotted floor in a specific spot for raising birds. The birds were grown under standard environmental conditions, as per the rearing guidelines of the Ross breed. On the primary days of chick arrival the temperature was 33 °C which was kept up until the end of the first week. Then the temperature was gradually reduced to 25 °C on day 22 and kept up with this level before the finish of the period. During the experiment, water and feed were given ad libitum and were free of coccidiostats. A standard commercial diet was utilized for feeding the birds. Toward the end of the second week, the birds were moved from the litter to the cages and randomly gathered in groups. The birds were isolated into four groups of 30 and five repeats of 6 inside each group. Group (A) takes a herbal mixture at a dose of 1 cc in 1 L of water for four days. Group (B) was treated with toltrazuril with 1 cc/litre for four days. Group (C) was experimentally infected with mixed *Eimeria* spp., but they did not have any treatment; this group was our positive control. Group (D) was not infected and was healthy throughout the experimental period; this was our negative control.

Weighing birds, measuring feed consumption, and calculating feed conversion ratio

The birds were weighed altogether until the fourteenth day. Toward the end of the second week, the birds were grouped, and the weighing procedure was performed for individuals of each group. Subsequently, the average weight of each group was calculated. The weighing of the birds went on until day 42 of the experiment.

Throughout the study, the amount of daily feed consumed in each replicate of the groups was recorded separately. By calculating how much meal was consumed and the expansion in bird weight during the week, the feed conversion ratio was determined.

Challenge birds using *Eimeria* oocysts

Combination of sporulated *Eimeria* oocysts was bought from the Faculty of Veterinary Medicine of the University of Tehran. A rapid slide test was taken from the prearranged combination, and a variety of *Eimeria* sporulated oocyst species was observed under the microscope. Species of this combination included 50% of *Eimeria tenella*, 25% of *Eimeria maxima*, and the leftover 25% had different species, for example, *Eimeria acervulina*, *Eimeria mitis*, and *Eimeria necatrix*. Each broiler in groups A, B, and C were challenged via oral gavage with 200,000 sporulated oocysts of the prepared *Eimeria* spp, on day 14 of age.

Registration of clinical symptoms and investigation of possible casualties

During the test period, birds in all groups went through everyday consideration for clinical signs, and the symptoms were recorded. Symptoms include reduced daily feed intake, lethargy and sleepiness of the birds, and diarrhea. In situations where fatalities were seen in the groups, the remains were necropsied straightaway, and the reason for death and injury were recorded.

Treatment using drugs

In the current experiment, on the fourth day of taking oocysts, specific symptoms of the infection like bloody diarrhea and weakness were seen in challenged groups, and treatment was started. The medicines-to the extent mentioned in the grouping part of the birds-were given to the birds of each group consistently for 28 days. The length of the treatment period is based on the producer's suggestion.

Calculation of output per gram (OPG) in different groups

According to the standard method of using the McMaster counting slide, the number of oocysts per gram of feces was counted (Haug et al. 2006; Pajić et al. 2018). On this matter, 3 g of feces from birds of each group were mixed with 42 ccs of water and shaken vigorously to obtain a uniform mixture. Then 15 cc of this mixture is centrifuged at a speed of 2000 rpm for 10 min, and in the next step, the supernatant is discarded while water and saturated salt are added to the formed sediment to bring the volume to 15 ccs. In the next step, we pour some part of it on the cells of the McMaster slide, and by placing the slide on a flat surface for 5 min, the oocysts have a chance to float. The oocysts are counted with a 10 × magnification of the compound microscope, and the average obtained from the two cells of the McMaster slide is expressed as OPG in that sample.

During the trial period, birds from each group were sampled, and fecal oocysts were counted on four different days: the first time on the fifth day after the challenge; The second time on the seventh day after the challenge; The third time on the ninth day after the challenge; The fourth time is the twelfth day after the challenge. The number of excreted oocysts in the feces of birds was recorded.

Table 1 Mean weight of birds in four groups of study during the 42 days

Group	First day	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Herbal mixture	48	171	408	645[a]	855[a]	1325[a]	1845[a]
Toltrazuril	48	171	408	650[a]	865[a]	1310[a]	1833[a]
Challenged and not treated	48	171	408	550[a]	751[a]	1259[a]	1655[a]
No challenge no treatment	48	171	408	670[a]	955[a]	1431[a]	1881[a]

Means denoted by different superscript letters show significant differences between groups in each column ($P < 0.05$)

Table 2 Weekly FCR values of birds of four groups during the study

Group	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Herbal mixture	2.71	2.62	3.1[a]	3[a]	2.23[a]	1.69[a]
Toltrazuril	2.71	2.62	3.5[b]	3.48[b]	2.31[a]	1.98[b]
Challenged and not treated	2.71	2.62	5.4[c]	5.43[c]	2.75[b]	2.42[c]
No challenge no treatment	2.71	2.62	2.3[d]	2.39[d]	1.4[c]	1.8[ab]

Different values indicate a significant difference between groups in each column ($P < 0.05$)

Lesion scoring

The descriptive method of Johnson and Reid (1970) was used on this matter (Johnson and Reid 1970). Based on the lesions observed in different parts of the intestine, a score of 0 (healthy) to + 4 (the most severe lesions) is considered. It should be noted that for scoring, four areas of the intestine, namely the beginning of the intestine, including the duodenum, the middle part of the intestine, including the jejunum and ileum, and the end of the intestine, including the colon and cecum were examined. In this study, lesions were recorded in four parts of the intestine, and then the average score of lesions in the studied groups was recorded. At the end of treatment, two birds from each replicate were slaughtered humanely, lesions of different parts of the intestine were recorded, and the average lesions in the carcasses were obtained.

Statistical analysis

The data on body weight, FCR, OPG, and LS parameters were analyzed by ANOVA and post hoc tests (SPSS® Software version 16). The discrepancy was considered statistically significant at the P-value < 0.05 for all the analyzed data.

Result

Weight gains

The average weight of birds in different groups during the breeding period (42 days) is given in (Table 1). Accordingly, the most noticeable weight gains were seen in the groups that didn't have challenges by *Eimeria* oocysts (D). They consistently had the most remarkable average weight during the period. The minor weight gain had a place with a group (C), which was not treated during the challenge. The weight contrast between the other treated group isn't noticeable. The critical thing to mention about coccidiosis is that this disease will decrease weight gain in meat herds, which causes significant economic damage. In the present experiment, group (D) gained weight more than the other groups, so the control and prevention of coccidiosis can reduce the damage caused by this disease.

Feed conversion ratio

The feed conversion ratio (weekly) results in different groups from the beginning to the end of the experiment are given in (Table 2). As can be seen in Table 2, the best results were seen during the rearing period–except on day 42—in group (D). The best feed conversion ratio on day 42 was observed in the group that received an herbal mixture. It is vital about the adverse effect of coccidiosis on the feed conversion ratio, which is very clear in the table above on day 21 and prompted a massive contrast in the feed conversion ratio of the group (D) with other study groups. Yet, with the use of medication in groups (A) and (B), the condition of birds has also improved and doesn't distinguish them from the unchallenged group.

Clinical signs and casualties

The birds were perfectly healthy on the day of feeding the oocysts and had no particular clinical problems. Stool consistency was also normal. Oocysts were fed on day 14 in groups (A), (B), and (C). Two days after the challenge,

Table 3 Lesion scoring of the intestine in different groups

Group	The proximal part of the intestine	The middle part of the intestine	caecum	The distal end of the intestine	Average lesion scores in each group
Herbal mixture	1	0	2.66	0	0.915[a]
Toltrazuril	0.66	0	2	0	0.66[b]
Challenged and not treated	1.66	0.66	3	0	1.33[c]
No challenge no treatment	0	0	0	0	0[d]

Different values indicate a significant difference between groups in each column ($P < 0.05$)

diarrhea was seen in the birds. Yet, the birds were clinically and appetizingly ordinary. On the fourth day after the challenge, chocolate-coloured dysentery and diarrhea were seen in various groups, and lethargy and anorexia were seen in birds. The decrease in feed consumption was also evident in the groups. Four days after the challenge and after observing signs, the treatment began in groups (A) and (B). Three days after taking the medication, the stool status returned to normal, and the overall state of the birds was normal.

In the group getting an herbal mixture, group (A), two deaths were observed, one before the beginning and the other passed on the third day of treatment. In group (B), three deaths were observed, one before the start of treatment and the other two related to one day after the beginning. In group (C), two deaths happened on treatment's second and third days. No losses were seen in the group (D). Necropsies were performed on casualties, and specific coccidiosis lesions were seen in all carcasses. The noticeable finding was connected with the caecum, where a severe lesion of + 4 was scored.

Lesions scoring

The best time to score lesions in coccidiosis is days 5 to 7 after infection. Based on lesions observed in different parts of the intestine. The average scores in each part are given in (Table 3). The results showed no lesion in group (D) was observed in the intestines. The group's (D) score was zero or equivalent to a healthy gut. Most injuries were observed in group (C) (a score of 1.33), which is reasonably expected. Notably, the intestinal lesions score of the group (A), compared to group (B), decreased and showed acceptable performance of an herbal mixture.

Counting the number of oocysts per gram of feces (OPG)

The results of counting excreted oocysts from birds in each group in 4 sampling stages (days 5, 7, 9, and 12 after the challenge) are presented in (Table 4). The results of counting the number of oocysts per gram of feces show that the herbal mixture has established a good performance and has reduced the number of oocysts per gram of feces. In the case of toltrazuril, drug use results have been satisfactory, and the number of oocysts excreted from birds has decreased with drug use. The point to consider in the results is that with drugs (herbal mixture and toltrazuril), a sharp decrease in fecal oocysts is observed on day nine after the challenge. But in the untreated group, a sharp reduction in excreted oocysts was observed on day 12 after the challenge.

Table 4 OPG values of different groups at 5, 7, 9, and 12 days post-challenge

Group	Day 5	Day 7	Day 9	Day 12
Herbal mixture	50000[a]	84000[a]	11650[a]	12200[a]
Toltrazuril	45000[b]	210000[b]	89000[b]	50000[b]
Challenged and not treated	32000[c]	150000[c]	132500[c]	32700[c]
No challenge no treatment	0[d]	0[d]	0[d]	0[d]

Different values indicate a significant difference between groups in each column ($P < 0.05$)

Discussion

Broiler herds, challenged by coccidiosis, experience a significant decrease in weight gain resulting in immense economic losses (Chand et al. 2016; Kadykalo et al. 2018). Observations of this study demonstrate that the birds in the non-challenged group had a distinguished difference in weight gain in comparison to those of other groups. Accordingly, the significance of managing and preventing coccidiosis damage can be inferred. Coccidiosis is an infectious disease that damages intestinal epithelial cells and results in dreadful hematochezia (Kostadinović et al. 2019). Common clinical signs such as anorexia, paleness, ruffled feathers, depression, and huddling together were observed during the present study, which complied with the ones witnessed in the studies by Dubey (2019) and Tanweer et al. (2014) (Dubey 2019; Tanweer et al. 2014). The results of this study demonstrate that the non-challenged group ingests more feed than other groups. These results are backed by Hashmi et al. (1994) and Tipu et al. (2002), who pointed out that coccidiosis infection causes a decrease in feed intake (Hashmi et al. 1994; Tipu et al. 2002). Considerable weight loss and reduced FCR are the consequence of all *Eimeria* isolates (Logan et al. 1993). The coccidia destroys the absorptive mucosal surface and competes for micronutrients resulting in the FCR reduction (Qaid et al. 2021). The consequence is a metabolic disorder, undesirably affecting nutrient utilization (Ali et al. 2019).A vast range of symptoms, from subclinical enteric infection to subacute mortality, can be induced by all 7 *Eimeria* species developing the chick's digestive tract (Adhikari et al. 2020). Multiple factors can affect the clinical outcome of coccidial infection, such as stress, environmental factors, host genetics, strains, congruent infections, infective dose, flock size, and *Eimeria* species (Nahed et al. 2022; Taylor et al. 2022). Based on the results provided by this experiment, birds fed with an herbal mixture had decreased oocyst counts. These results comply with former studies that used essential oil comprising garlic in broilers, in which fecal oocyst counts were remarkably decreased (Abou-Elkhair et al. 2014; Kumar et al. 2022; Sidiropoulou et al. 2020). Phytogenic supplementation (*Artemisia annua*, *Quercus infectoria,* and *Allium sativum*) left a crucial impact on oocyst counts. The phenolic compounds tested in the herbal mixture might cause lower oocysts to count in the infected group and be treated with an herbal mixture. Phenols can react to cytoplasmic membranes and modify cations' permeability, disrupting vital processes in the coccidian cells and causing their death (Sikkema et al. 1995). In addition, it is fair to claim that the organosulfur compounds that existed in *A. sativum* are the highest significant contents in charge of a major part of their pharmacological impacts (Sadr et al. 2022). Amid the mentioned biological active compounds, DTS, Allicin, ajoene, and allyl methyl sulfide have been proven to be the primary origin of antiprotozoal, antifungal, antiviral and antibacterial effects of *A. sativum* sequentially (Mikaili et al. 2013; Yadav et al. 2020). In *A. sativum*, a specific molecule called Allicin provokes immunity by extending profiling antibody response that straightaway destroys the sporozoites and has antioxidant and antiparasitic activity (Asghar et al. 2020). Allicin is easily permeable through the cell membrane and is suggested to use its activities either via inflicting oxidative damage to the cells or through antiproliferative action (Liu et al. 2021). The primary antimicrobial activity of Allicin is because of its chemical response with thiol groups of diverse enzymes, e.g., RNA polymerase, alcohol dehydrogenase, and thioredoxin reductase, which affect the vital metabolism of cysteine proteinase activity involved withinside the virulence of parasites (da Silveira Deminicis et al. 2021; Mohanad et al. 2019). In addition to its particular response to the free sulfhydryl group present in the parasite's active site of cysteine proteinase (Kothari et al. 2019). Mortality was drastically reduced in the garlic-protected group, and comparable efficacy was recorded by Abu-Akkada et al. 2010 (Abu-Akkada et al. 2010). *Allium sativum* has also been known for enhanced production of white blood cells, increased phagocytosis of infected organisms, and antibodies (Khan et al. 2012; Kim et al. 2013). Based on studies, the most vital product obtained by elimination from crushed fresh *A. sativum* is thiosulfinates, which is a volatile sulfur compound (Lawson 1996). The amount of sulfur content of the *A. sativum* weighs about 1% of its dry weight (Pentz et al. 1990; Ueda et al. 1991). Based on the current study, *Eimeria* species cause degenerative modifications in the positive control group. In contrast *A. annua*, *Q. infectoria,* and *A. sativum* supplementation reduced the intestinal lesions. Data obtained in the present experiment is supported by the result of the study by Gotep et al. (2016) that indicated the addition of garlic to feed broilers infected with coccidiosis leads to the maximum volume of crypt and villi of the small intestine (Gotep et al. 2016). Artemisia is a vast, various genus of herbs with two hundred to four hundred species belonging to the family *Asteraceae* (Ghafouri et al. 2023). The most remarkable species reported for its antiparasitic actions is *A. annua* (Abbas et al. 2012). Artemisinin's suggested mechanism of action includes cleavage of endoperoxide bridges by iron-producing free radicals (aldehydes, dicarbonyle compounds, hypervalent iron-oxo species, and epoxides), which destroy biological macromolecules triggering oxidative stress in the cells of the parasite (Jiao et al. 2018). In addition, *A. annua* contains many phenolic compounds, flavonoids, and phytochemicals which can support broilers to take up vast amounts of nitrogen and keep commensal microflora (Cobaxin-Cárdenas 2018). It can also cause the decrease of pro-inflammatory factors caused by immunological responses to *Eimeria* spp. and the parasite (Muthamilselvan et al. 2016). Oh et al. (1995) showed the first trial to assess the anticoccidial effect of *A. annua* extracts against *E. tenella* in broilers, and the herbal compound presented great anticoccidial action in terms of reduced lesion scores, improved weight gains, and improved feed conversion ratio (Oh et al. 1995). Allen et al. (1997) describe an unusually anticoccidial activity of *A. annua* against *E. tenella* (Allen et al. 1997). Arab and colleagues (2006) described OPG decreases of *E. acervulina* reaching from 90 to 95% follow-on from adding two different doses of pure artemisinin, and it recommends that artemisinin be able to cause a significant decrease in OPG, even under a serious challenge (Arab et al. 2006). The phytochemical experiments that held so far about *Q. infectoria* galls have discovered the presence of amentoflavone hexamethyl ether, anthocyanins, tannic acid (19.9%), syringic acid, isocryptomerin, starch, gallic acid (8.75%), essential oils, methyl-betulate, ellagic acid, polygalloyl-glucose,methyl-oleanate, and hexagalloyl-glucose (Sundar et al. 2017). Extracts of *Q.*

infectoria have potent antioxidant activities and free radical scavenging (Arina and Harisun 2019). The extract can chelate metal ions that catalyze the generation of oxidants and protect lipids and proteins against oxidative damage (Tonda et al. 2018).

Current research demonstrates that *Artemisia annua*, *Quercus infectoria,* and *Allium sativum* represent excellent protection against coccidiosis in broilers.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed**.**

Consent to participate Not applicable.

Consent for publication Not applicable.

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