In vitro study of curcumin calcium carbonate phosphate nanoparticles (Curcumin-NPs) impacts on the meriz goat’s coccidian oocysts

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Abstract

Nanoparticles biosynthesis has an essential and increased role in delivering medical compounds. Calcium carbonate phosphate nanoparticles (CaCO₃-NPs) were prepared as a stabilized amorphous and incorporated with herbal curcumin extract as an anticoccidial agent in vitro. CaCO₃-NPs were tested against local meriz goat coccidian oocysts. Concentrations were used 2, 4, 8, 16, 30 and 50 mg/ml shows oocysticidal effects and sporocystidal effects at concentration of 100, 200, 400, 800 and 1000 µg. Sporulation inhibition assay was used for 24 and 48 hours. Results of significant oocysticidal effect were seen to inhibit in the concentration of 30-50 mg/ml and able to inhibit the sporulation of meriz coccidian parasite oocysts at a rate of 92.54±3.51%. The sporocysticidal effect was also significant with a curcumin nanoparticles concentration of 400-1000 µg/ml with a rate of 98.1±2.11%. The stability of prepared curcumin nanoparticles was examined against various pH levels 4.01, 7, and 9.21 at multiple temperatures 4, 25, 60, and 100°C. Investigation after 1, 6, 12, and 24 hours of treatment occurs according to various treatments. Stability was assessed by spectrophotometric indicated significant reductions for pH 4 and 9 after one hour of treatment and at the temperature of 60°C and 100°C after 12-24 hours of treatment. These results reflect promising hopes of exploiting CaCO₃ curcumin nanoparticles to eradicate coccidiosis as they are composed of and prepared from natural substances.

Keywords: Nanoparticles, Curcumin, CaCO₃, Coccidia, Meriz goats

Introduction

Nano-biotechnology is a division of science that is exploited to enhance the efficacies of nanoparticles (NPs) for various applications of therapeutic drug categories by using numerous techniques and methods to study, design, and fabricate substances at atomic and molecular levels. The original concept of investigating ingredients and biological systems at the nanoscale went back to more than 40 years ago when Richard Feynman presented a lecture in 1959 at the annual meeting of the American Physical Society at the California Institute of Technology (1). Recently, nanotechnology can include research, and advanced work on materials and species at length scales 1 to 100 nm. Nanotechnology is vital to biology since several biological parameters have molecular structures at the nanoscale levels. These parameters cover a wide variety of basic structures, such as proteins, polymers, carbohydrates (sugars), and lipids, which have a countless variety of physical, chemical, and functional properties (2,3). Nanoparticles are classified into diverse classes, for example, inorganic nanoparticles, organic nanoparticles, ceramic nanoparticles, and carbon-based nanoparticles. The inorganic nanoparticles are more sub classified into metal...
and oxide nanoparticles, such as oxide. Correspondingly
carbon base nanoparticles are classified into fullerene,
carbon nanotubes, graphene, carbon nanofiber, and carbon
black nanoparticles, which are further classified based on
dimensions, such as one-dimension nanoparticles, two-
dimension nanoparticles, and three-dimension nanoparticles
(4,5).

Recently, tissue-specific criteria have been utilized to
characterize homing peptides and prepare nanoparticles to
target drug delivery toward specific body organs. This
strategy decreased adverse treatment complications on
unintended tissues (6). Scientists have also prepared
nanoparticles to increase the effectiveness of applied
medications and minimize off-target effects (7,8). In
addition, drug-loaded nanoparticles, for instance,
ilosomes, were synthesized and designed to bind to tissue-
specific epitopes to release incorporated medication at the
intended organs, such as the placenta (8,9). However, these
nanoparticles may exert certain complications and inhibit
specific physiological functions (10). CaCO₃ has to be
significantly considered in research due to its rewards,
including affordability, low toxicity, biocompatibility, 
cytocompatibility, pH sensitivity, sedate bio-degradability,
and environmentally responsive materials (11,12).
Additionally, modification of NPs for therapeutic purposes
has concerned considerable attention by researchers to
improve the solubility, stability, circulation half-life, and
biodistribution of the encapsulated agent (13,14).

Turmeric Curcumin (C. longa) as an old-style medicinal
herb was castoff for various determinations for refining of
overall health and also as a medication in many cases
(15,16). Curcumin was intermittently used in animal health
due to its growth increment, antimicrobial, anti-
inflammatory, and neuroprotective effects (13,17). The
dried turmeric rhizome consists of 3-6% terpenes and
terpenoids, 6-8% proteins, 6-10% fats, 60-70% carbohydrates, and 3-6% fibers (18). Curcumin or
diferuloylmethane [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione],
diferuloylmethane, diferuloylmethane, natural yellow 3 with known structure. It
is a hydrophobic and polyphenolic complex extracted from
Curcuma longa (19). Curcumin is also used to reduce
parasitic coccidiosis disease's pathological manifestations
in animal and poultry industries (20). Many studies on
curcumin were done either in vivo or in vitro (20,21) using
the direct raw or extract materials of the compound (13,22)
as well as in ex vivo (23) to ensure medicinal application of
these effects.

Previously (24), local meriz goats (Capra ibex) showed
a high infection rate 37.67% with six coccidias of genus
Eimeria of various species E. alijevi, E. christenseni, E.
caprovina, E. minasensis, E. megaembryonica, E.
ninakohlyakimovae, and E. megaembryonica, this infection
rate necessitated some suggestions and trials to work on the
prevention, treatment, and reduction of infection rates via
using nanoparticles as a novel approach to improving the
health and welfare of meriz goats, which are considered
species of the most economically significant animals.

In this study, CaCO₃ - curcumin NPs were used to
investigate their effects on the oocysts (as Oocysticidal)
and the sporocysts (as sporocysticidal) of coccidian parasite
of genus Eimeria that infected local meriz goats in vitro.
These effects of CaCO₃ - curcumin NPs will be examined at
various levels of concentration, time, pH, and temperature.

**Materials and methods**

**Herbal extract of curcumin**

Curcumin of 99% purity was purchased from (Sigma-
Aldrich), which contain Curcumin, ≥94% (curcinoid
content), ≥80% (Curcumin) according to product
comparison guide of the company.

**Curcumin nanoparticles preparation**

CaCO₃ nanoparticles were provided from a local
chemical store, and the amorphous phase was prepared
according to Rao et al. (12,25). Briefly, the aqueous
solutions of CaCl₂, NaCO₃, Na₂HPO₄,12H₂O) with
deionized distilled water 100 ml were used for each. CaCl₂
(10 mM), 0.07419X2 g of NaCO₃, Na₂CO₃ (0.107419X2g)
and 0.1074219X2g of Na₂HPO₄,12H₂O were dissolved in 20
ml of deionized distilled water. Sodium Phosphate hydrate
solution was added as a drop by drop to CaCl₂. All
substances in the solution were allowed to mix for 15
minutes at 30°C. Curcumin solution (w/v) preparations in
proper concentrations at 2, 4, 8, 16, 30 and 50 mg per ml
for oocysticidal and 100,200,400, 800 and 1000 μg for
sporocysticidal effects, were prepared with sterilized
deionized distilled water. These solutions were added
simultaneously into the CaCO₃ solution.

**Oocysticidal and sporocysticidal effects**

Oocysts of a coccidia parasite were isolated from local
meriz goats as previously described (24). Five grams of
fresh fecal sample were collected in sporadic containers,
then transferred to the lab and mixed with distilled water
v/v (1X10). The sample was sieved with four layers of
sterilized cotton gauze and washed three times with PBS.
After that, 0.15 ml was placed on a clean glass slide, the
coverslip was adapted, and total coccidia oocysts were
counted. One hundred oocysts were used for each treatment
to estimate the oocysticidal effect by preventing further
development and sporocysticidal effect by preventing the
formation of sporocysts and sporozoites inside it, and
followed by a simple compound microscope (X40). This
procedure was previously performed in the poultry type of
coccidia (22,26). Then, the proper concentration of
curcumin- NPs was used according to the planned time and
pH described above.
Stability of curcumin-NPs
Preparation was tested against various pH in different standard buffer solutions at pH 9.21, pH 7, and pH 4.01. Stability was tested after 1, 6, 12, and 24 hrs. by screening change in the spectrum of a spectrophotometer at 424 nm. This is the first study that examines the stability of curcumin-NPs using this method. Thus, it is considered a novel method for this type of NPs.

Thermal stability of prepared curcumin - NPs
It is examined at 4, 25, 45, 65, and 100°C for 1, 6, 12, and 24 hrs. (For boiling, just 1, 2, and 3 hrs. were used). The screening was done as described above for both pH and temperature with the aid of a spectrophotometer according to protocols used in previous studies (12,25).

Statistical analysis
All tests were done f to minimize possible reading errors. Statistics of Chi-square for both, oocysticidal and sporocysticidal effects was adapted, and ANOVA was used for pH and thermal stability with the aid of Duncan’s multiple range test by using PROC GLM procedure of SAS (27).

Results
According to previously published scientific research, the CaCo3 - curcumin NPs were prepared successfully. It shows well and observed effects according to the following sequence of current treatments against the local meriz Eimeria coccidia parasite (Figure 1).

Table 1 explains the results of the In-vitro oocysticidal effects of curcumin-NPs against local meriz (Capra ibex) coccidian parasites (temp/time/concentration). The data showed significant variations according to used p values (P=0.05 and P=0.01). In 2 mg/ml concentration, it is clear that sporulation inhibition rate arranged from 25.7 to 37.5% for various temperatures and times. In a concentration of 4 mg/ml and above, the results exhibited a significance from 12 hours at 39°C and up. These results reflected that prepared curcumin-NPs effectively inhibited the sporulation process of coccidia oocysts in vitro according to various concentrations at different temperatures and rates of 25.7-88.3%.

Table 1: Curcumin-NPs in vitro oocysticidal effects against local meriz coccidian parasites

<table>
<thead>
<tr>
<th>Curcumin-NPs (mg/ml)</th>
<th>Temperature 25°C (hr.)</th>
<th>Temperature 39°C (hr.)</th>
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<tr>
<td></td>
<td>12</td>
<td>24</td>
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<td>2 (mg/ml)</td>
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<td>30 (mg/ml)</td>
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<td>50 (mg/ml)</td>
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<tr>
<td>Control negative (2% Pot Dichromate w/v)</td>
<td>21a</td>
<td>1**</td>
</tr>
<tr>
<td>Control positive (0.5 mg Amprolium/ml)</td>
<td>95+</td>
<td>99+</td>
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</table>

1= Vs. 100 oocystes / ml // Normal rectal goat temp is 39// By Chi square. a=Is significance in lower than others (c, b and d). b= Is significance in P=0.05 to others (a and b). c= Is significance in P=0.05 to b, and in P= 0.01 to a. d= Is significance in P=0.01 to all others (a, b and c). + = Is none significance among all these treatment vs. control positive. ++=Is significance (P=0.05 and P=0.01) for all treatments vs. control negative.
The NPs were analyzed with no significant variation (P=0.05) vs. a and c.

Table 3 shows the curcumin-NPs stability ratio versus various pH and temperatures by using absorbance at 424 nm through the release method. The NPs were stable, and the data has revealed a lower releasing ratio 13%, 5%, and 10% from curcumin at 4°C at various pH 9.21, 7, and 4.01 with no significant variations correlated to the prepared concentration. Releasing ratio started to elevate with an increasing temperature 25°C and above. The pH played a major role in releasing curcumin as the temperature raised. Significant (P=0.001) variation was observed from 4°C to 100°C. The releasing ratio was seen to be stable and acceptable 13-45% until 60°C for various pH readings. The data from this study shows that the curcumin-NPs preparations were stable at the range of releasing time in about 13-94% according to various temperatures and pH.

Discussion

Coccidiosis is a significant protozoa disease of many animals such as sheep, goats (28), camels (29) as well as poultry of various species (30,31). Herbal pharmacological medicine shows an attractive category in control of it by inhibiting its life cycle. Effectivity of inhibiting sporulation process of coccidia oocysts in vitro was observed by Manafi (32), who is showed that herbal medicines in chicken (in vivo) leads to a reduction in the clinical signs of coccidiosis by degeneration of schizonts of Oocysts, which indicates a decrease in the sporulation of the parasite. In addition, Kheirabadi et al. (33) have used herbal medicines in chicken to reduce sporulation and oocysts per gram of coccidia. Both studies were conducted in vivo, however, in chicken, the herbs are not specified, and they are unrelated to Curcumin-NPs.

Furthermore, Yadav et al. (21) have discussed the influence of different doses of curcumin herbs on many aspects of chicken performance, including their effects against coccidia. The researchers used an in vivo challenge test with food supplemented with extract of curcumin, which was obtained from the herbal plant Curcuma longa. In their study, they have observed similar activities. Similar effects were seen in previous studies on poultry (22,34), but not for in vitro work, animal species such as meriz goats, using different doses, times, and temperatures.

These bioactivities of curcumin could be attributed to the components that have been derived from a rhizome of turmeric (Curcuma longa), which may interact with several cellular or molecular targets (such as many cellular communication molecules as NF-Kβ, JAKs/STATs, MAPKs, TNF-γ, IL-6, PPARγ, and TRPVL1). Moreover, the antitumor, antimicrobial, and wound-healing effects are due to curcumin bioactivity (35). The suppression of gluconeogenic gene expression could be the cornerstone of this action for all eukaryotic cells (36). Also, the ability of curcumin to modulate the functions of multiple signal
transductions is linked with the attenuation of acute and chronic diseases (37).

The preparation of nanoparticles from natural resources has shown specific medicinal effects that can be useful in treating specific kinds of pathological disorders (38,39). Many studies show an agreement that curcuminoids as bioactive compounds of C. longa L. can remain stable while exposing to heating treatment during the preparation process (40). Furthermore, Oshi et al. (41) found that the release rate of curcumin from the core-shell nanoparticles was low at a pH mimicking the stomach and small intestine. In contrast, it was higher at a pH mimicking the colon and not dissolved in the upper GIT, resulting in the high availability of the drug in the colon for therapeutic activity. In addition, Sun et al. (42) clarified that CaCO3 nanoprobe resists low pH effect in vitro. Previous investigation (10) on other preparations of nanoparticles displayed that they can remain stable for 28 days, but these studies have used 4°C as the only storage temperature. Researchers have also demonstrated that nanoparticle preparations show efficient releasing properties as in this study regarding the incorporated materials at the site of action (8,9) or different NPs compounds used (12,37).

Conclusion

Curcumin- NPs prepared in the current study showed influential oocysticidal and sporocysticidal effects on coccidia oocysts In -vito. These findings are considered promising strategies to be applied in the field as the compounds are derived from natural sources, and they are reliable to be exploited for the prevention and control of coccidia and to be prepared as a pharmaceutical formulation. Regardless of complex techniques used in the preparation and investigation of nanoparticles, the current study revealed that spectrophotometric methods are applied easily in the study stability of nanoparticles, which may be essential for further research when field application may be investigated lately.

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Conflict of interest

The authors declares that there are no any conflicts of interest with regards to the manuscript

References


