10.21608/avmj.2025.319617.1393 Assiut University web-site: www.aun.edu.eg

EFFICACY OF NANOEMULSION OF *YUCCA SHIDIGERA* EXTRACT IN CONTROLLING *EIMERIA TENELLA* INFECTION IN BROILERS

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Received: 16 October 2024; **Accepted:** 6 March 2025

ABSTRACT

There is scientific evidence that nanotechnology might increase the stability, delivery, and cellular absorption of medications. Therefore, this study was designed to investigate *Yucca shidigera* (YS) extract nanoemulsion efficacy in the prevention and treatment of coccidiosis in broilers. YS extract 5% was formulated as nanoemulsion (YSN) and then characterized by using zeta sizer and zeta potential. Six groups of 35 chicks/group, one-day-old each, were categorized as negative control, positive control, 2 treatment groups of YS and YSN, and 2 prophylaxis groups of YS and YSN. The treatment groups were administered YS and YSN at day 6 post-infection, while the prophylaxis groups received YS and YSN 5 days before infection. The YS and YSN doses were 100 mg/L in water. All groups except the control negative were orally infected with about 25 × 10^3 *Eimeria tenella* sporulated oocysts per chick at day 23 of the chicks' age. Both treatment and prophylaxis groups revealed a significant decrease in oocyst count, rapid recovery from the disease, and better body weight gain than the infected untreated group. The blood chemistry and hemograms in the treatment and prophylaxis groups showed no significant difference from the control infected untreated group. The pathological pictures showed a lower number of parasitic stages in the cecal tissue in the treated groups, compared to the control-infected untreated chicks. In conclusion, YS and YSN mitigate the pathogenicity of *E. tenella* through a reduction in the oocyst count and improve body weight gain. The present study reported no significant difference between YS and its nanoemulsion form.

Keywords: Chicks, nanoemulsion, *Yucca schidigera*, *Eimeria tenella*, body weight, biochemical parameters.

INTRODUCTION

Coccidiosis is a protozoan disease that affects chickens; it is caused more frequently by various Eimeria species, including *E. tenella, E. acervulina, E. necatrix, E. brunetti, E. maxima, E. mitis, and E. praecox* (Shirley, 1986). Coccidiosis produces economic losses in the chicken industry due to significant mortality and weight loss (Williams, 2002; Nahed *et al.*, 2022). Based on updated information from veterinarians, farmers,

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production experts, and health professionals, the Williams model estimates that coccidiosis in chickens cost the UK approximately £99.2 million in 2016. Applying this model to data from the United States, New Zealand, Nigeria, Brazil, India, Guatemala, and Egypt suggests a global cost of about £10.4 billion at 2016 prices, and this is equivalent to £0.16 per chicken produced (Blake *et al.,* 2020). The primary pathogenic consequences of coccidiosis are oxidative stress and an immunological anti-inflammatory response (Nahed *et al.,* 2022; Rizwan *et al.,* 2022; Gul and Alsayeqh, 2023). *Eimeria tenella* attacked the caecal lining epithelium of chickens, producing bloody diarrhea, reduced feed intake, losses in body weight, and significant mortality (Zhou *et al.,* 2013; Pop *et al.,* 2015). Thus far, the recurrent use of chemoprophylaxis and anticoccidials led to the emergence of drug resistance (Abbas *et al.,* 2008).

Yucca schidigera is a plant species that grows widely in the Americas. The extract contains numerous phytochemicals, including resveratrol, glycol, polyphenols, and steroid saponins (Cheeke *et al.,* 2000; Oleszek *et al.,* 2001). The inclusion of 120 mg/kg Yucca extract to layers' diet improved egg weight and feed efficiency, lowered cholesterol levels in serum and yolk, and reduced the growth of Escherichia coli (Wang *et al.,* 2011). YS has antioxidant (Farag *et al.,* 2018), anti-inflammatory, and immunomodulatory effects (Gupta *et al.,* 2014). YS extract is considered a major source of saponins that interacted with cholesterol formation in the cell membrane of the protozoal developmental stages, prevented its development, and killed it **(**Wang *et al.,* 1998**)**. Yucca may enhance antioxidant levels and reduce the heat stress reaction in developing broilers by controlling the expression of genes that sense body temperature in the hypothalamus (Luo *et al.,* 2022). Additionally, feeding broilers supplementation with yucca increased feed intake, most likely via reducing circulation of cholecystokinin (CCK) in developing broilers under high ambient temperatures (Luo *et al.,* 2022). Kozłowski *et al.* (2022) reported the anticoccidial effect of YS by decreasing oocysts count and improving the body performance of the treated chickens.

Nanotechnology's importance in veterinary medicine is due to the enhancement of medicinal products delivery. Nanoemulsion carrier systems are utilized in pharmaceuticals to deliver bioactive compounds poorly soluble in water (McClements, 2012). Nanoemulsions increase the bioavailability and bioactivity of products (Acosta, 2009). Chitosan nanoencapsulation enhances the health benefits of essential oils of thyme, cinnamon, and mint in broilers (Nouri 2019).

Therefore, the current study aimed to investigate the efficacy of *Yucca shidigera* extract in nano emulsion formulation either in treatment or prophylaxis of *Eimeria tenella* infection in experimentally infected chicks.

MATERIALS AND METHODS

1. Ethics statement

The experiments were carried out in compliance with the protocols and ethical standards set out by Beni Suef University's Faculty of Veterinary Medicine in Egypt (2017-BSUV-11).

2. *Yucca shidigera* (YS) extract, GC-MS and its nanoemulsion 5 % (YSN)

YS extract was supplied by ABChem Egypt. This extract was analyzed, and the GC-MS confirmed its content of (30%) saponins. The prepared of YS emulsion had a concentration of 5% depending on a previous work (Kozłowski *et al.,* 2022). The YSN was prepared in a 100 mL glass baker; 16.7 mL of YS extract (30 %) was

dissolved in tween 80 and 10 mL of monopropylene glycol using a magnetic stirrer for 10 min at 500 rpm, then complete with distilled water up to 100 mL with stirring another 10 min until the YS extract completely dissolved. The obtained solution was then sonicated in a sonicator for 5 min according to El-Sawah *et al.* (2024) for nanoemulsion formed. Charact-erization of the formed nanoemulsion was carried out using a Malvern Zeta sizer nano series instrument (Ibrahium *et al.*, 2022). This YSN 5 % saponins end product was administered as anticoccidial in drinking water one mL per liter drinking water, equivalent to 0.05 g saponins per 1 L (Kozłowski *et al.*, 2022)

3. Eimeria tenella isolate

This isolate was provided by the parasitology department of Beni-Suef University's veterinary medicine college. Our strain was originally propagated in 5 chicks to yield enough oocysts for our investigation. The birds were euthanized eight days after inoculation, and the ceca contents were collected. The collected oocysts were concentrated, sporulated in 2.5% potassium dichromate, examined microscopically for morphology and sporulation, then kept in a refrigerator (2–5 °C) until used in the experimental infection (Ewais *et al.*, 2023).

4. Experimental chicks

210 one-day-old chicks of the Cobb breed were reared in the lab house of the Poultry Disease Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. The raising system was made up of individual pens with floors that were gated and openings. The feed ratios are divided into three different energy and protein concentrations: 23% starter, 21% growth, and 19% finisher. The feeding rations were made using a particular type of commercial feed free of antibiotics and anticoccidials to ensure that the outcomes were unaffected by factors other than materials that had undergone experimental examination. A 24-h. light plan permits unrestricted consumption of food and beverages. All chicks were fecally examined weekly till the day of infection.

5. Experimental design

The experiment involved 210 one-day-old chicks, which were raised until they were 35 days old. The chicks were divided into 6 groups, with each group consisting of 35 chicks (5 replicates of 7 chicks each). There were a control negative uninfected untreated group and a control positive infected untreated group. YS 5% extract and its nanoemulsion form (YSN 5%) were tested in two strategies; treatment and prophylaxis. The treatment groups, YS and YSN were administrated the treatments when the clinical signs appeared on day 4 post infection. The dose of YS and YSN was 100 mg/L in water. However, the prophylaxis groups received YS and YSN five days before infection and continued five days post infection (Elsawah et al., 2021). The chicks in all groups at the age of 23 days, except for the control negative group, were infected orally using pasteur pipette with 25×10^3 sporulated oocysts of *E. tenella* and monitored daily for observation. For each group, clinical signs, lesion score, body weight, feed consumption, feed conversion rate, and general health status were noted. At days 6^{th}, 9^{th}, and 12^{th} post infections, five birds (a bird / replicate) from each group were randomly chosen, humanely sacrificed, and blood samples were collected. The ceca were taken for histopathology, and the blood samples were taken for hematological and biochemistry testing. From day 6 to day 10 post infection, five random fecal samples were taken daily. These samples were examined to count and calculate the daily rate of oocyst shedding.

6. Evaluation of the efficacy of YS and YSN as anticoccidial drug.

6.1. Clinical and parasitological parameters

The clinical signs of cecal coccidiosis, mortality rate, bloody diarrhea score, and cecal lesions score were reported

(Morehouse and Barron 1970; Johnson and Reid 1970). Five pooled fecal samples from each group's replicates were collected every day (Lillehoj and Ruff 1987). The fecal samples were well mixed in plastic cups before being processed. One gram of the combined faecal sample was ten-dilution with saturated salt solution, and the oocysts were counted on a McMaster slide. The daily number of oocysts was measured in grams of feces three times for each group (Aboelhadid *et al.,* 2019). This procedure was carried out every day from day 6 till day 10 post infection.

6.2. Performance indicators

The growth rate and feed conversion ratio were measured by weighing each group's individual birds at the beginning of the experiment and repeating the weigh-in every week (Ma *et al.,* 2011). The feed conversion ratio (FCR) was determined using the following formula, according to Voeten *et al.* (1988): FCR = total weight (g) of feed consumed by each group of birds over a certain period / total weight gain (g) of the same birds, even the diseased birds throughout the same period. At the end of the experiment, a carcass cut was done for each group.

6.3. Cecal histopathology examination

Histopathology was performed on five caecal tissue samples from each group for detection of parasitic stages and changes in the tissues. This process involved collecting small pieces of caecal tissue, which were then fixed in 10% formalin, washed overnight in tap water, dehydrated, embedded in paraffin wax, and sectioned into 5µm thick slices. The sections were rehydrated through increasing concen-trations of ethyl alcohol before being stained with hematoxylin and eosin (H&E) (Bancroft and Gamble 2008).

6.4. Blood chemistry parameters

Five blood samples were obtained from each group, and the serum was prepared by centrifuging the samples at 3000 rpm for 10 min. A full automation system can assess the liver enzymes and renal enzymes (Fuji Dry Chem NX500 automatically). According to Reitman and Frankel (1957), liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. Moreover, kidney functions were tested by measurement of creatinine and blood urea nitrogen (BUN) (Henry, 1974).

6.5. Hematological parameters

Blood cells count was done using CBC automated veterinary analyzer. Five blood samples were taken from each group on the sixth, ninth, and twelfth days after infection (El-Madawy *et al.,* 2022).

7. Statistical analysis

The Shapiro-Wilk test was performed to examine if the data was distributed normally. To determine differences between groups, data were analyzed with an ANOVA and a Tukey multiple range test (Graphpad Software, San Diego, CA). The results were reported as means and standard deviations. Statistical significance was defined as a probability value of 0. 001 (P≤ 001).

RESULTS

1. *Yucca schidigera* extract nanoemulsion (YSN) characterization

YSN exhibited a negative charge on its outer surface (-0.141 mV) and had a mean hydrodynamic particle size of approximately 356 nm. The polydispersity index (PDI) was 0.669, indicating a narrow size distribution of the droplets, as shown in Figures 1 and 2. This low PDI value (<1.00) reflects the uniformity in droplet size.

2. Clinical signs and oocysts count

On the day of challenge, all birds in all groups showed similar parameters, feed intake, feed conversion and daily body

weight, to avoid any interference of any factors with the trial results. The control negative uninfected chicks showed no clinical signs of cecal coccidiosis. However, typical clinical signs of coccidiosis appeared in the control infected untreated group: bloody diarrhea, ruffled feather, and feeding rate reduction, and decreased vitality. These clinical signs also appeared in all groups, either prophylaxis or treatment groups, especially on days 6 and 7 days' post infection. These clinical signs declined sharply in prophylaxis groups and gradually in treated groups (Table 1). Regarding the mortality rate, the control negative group showed no deaths. However, the control infected untreated chicks revealed the highest mortality rate (42.85%). YS and YSN treated groups showed a mortality rate lower than the control infected group. YS and YSN in prophylaxis and treatment trials showed lesion scores significantly lower than control infected untreated (Table 1). Furthermore, YS and YSN in prophylaxis and treatment trials showed significantly lower oocysts count than the control infected untreated (Table 2).

Table 1: Bloody diarrhea score and mortality rate in the different experimental groups.

Day/Group	6th dpi	7th dpi	8th dpi	9th dpi	Mortality rate
Negative control, uninfected untreated	0	0	0	0	0
Positive control, infected untreated)	++++ (score 4)	++++ (score 4)	+++ (score 3)	++ (score 2)	15 (42.85%)
Y. shidegra extract 5% (YS) treatment	++++ (score 4)	+++ (score 3)	++ (score 2)	+ (score 1)	13 (37.14%)
Y. shidegra extract 5% nanoemulsion treatment	++++ (score 4)	+++ (score 3)	++ (score 2)	+ (score 1)	12 (34.29%)
Y. shidegra extract 5% prophylaxis	++++ (score 4)	+++ (score 3)	++ (score 2)	+ (score 1)	13 (37.14%)
Y. schidigera extract 5% nanoemulsion prophylaxis	++++ (score 4)	+++ (score 3)	++ (score 2)	+ (score 1)	12 (34.29%)

Bloody diarrhea score in treated and untreated groups: + means 0- 25% blood in the feces, ++ means 50% blood in the feces, +++ means 75% blood in the feces and ++++ means 100% blood in the feces. DPI day post infection

Table 2: Oocyst count of different groups during the experiment.

	Oocysts count (10^3)			
Group / days	**DPI 7**	**DPI 8**	**DPI 9**	**DPI 10**
Control negative uninfected untreated	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control positive infected untreated)	163 ± 37.5	318 ± 50.8	196 ± 24.5	104 ± 18.9
Yucca shidegra extract 5% (YS) treatment	102 ± 22.6*	110 ± 9.85*	62.0 ± 9.16*	37.3 ± 10.2*
Yucca shidegra extract 5% nanoemulsion treatment	104 ± 15.0*	117 ± 17.6*	65.0 ± 16.6*	34.3 ± 6.11*
Yucca shidegra extract 5% prophylaxis	103 ± 10.4*	171 ± 23.6*	72.0 ± 8.18*	51.0 ± 9.00*
Yucca schidigera extract 5% nanoemulsion prophylaxis	108 ± 13.0*	97.0± 14.9*	52.7 ± 13.6*	41.0 ± 9.85*

(*) significant for negative control. DPI day post infection

3. Performance and feed conversion rate

At the end of the experiment, 35 days of chicken age, the control negative uninfected birds showed the best body weight. However, YS and YSN groups showed significantly better results than the control positive infected untreated (Table 3). The best FCR was recorded in the control negative group. The worst FCR was recorded in the control infected untreated group (35-day-old broilers). YS and YSN recorded significantly better than the control positive infected untreated (Table 3).

4. Chicken carcass cuts

The control negative chicks recorded the highest whole chicken weight (2033 g), with noticed improvements in all chicken cuts. The control infected untreated group recorded the worst whole chicken weight (1525 g) and retardation of all cuts weight about 25%. YS and YSN forms in both trials reported success in recording final end products about 1800 g with increased about 15% more than infected untreated group, breast quarter and leg quarter that represent the most important cuts recorded a significant increased weight in YS and YSN groups about 20: 25% more than infected untreated group (Table 4).

Table 3: Body weight and feed conversion ratio at day 35 of chickens' age, the end of the experiment

Groups	Body weight (g)	FCR	Parasitic stages in the tissues
Negative control, uninfected untreated	2033 ± 156	1.56 ± 0.01	0.0
Positive control, infected untreated)	1527 ± 72.5*	2.09 ± 0.21	67.5±5.5
Yucca shidegra extract 5% (YS) treatment	1805 ± 124	1.90 ± 0.07*	19±4
Yucca shidegra extract 5% nanoemulsion treatment	1712 ± 103*	1.85 ± 0.03*	16.5±4.5
Yucca shidegra extract 5% prophylaxis	1823 ± 413	1.83 ± 0.06*	16.2±3.6
Yucca schidigera extract 5% nanoemulsion prophylaxis	1850 ± 157	1.79 ± 0.11*	14.4±4.8

(*) significant for negative control

5. Cecal histopathological

In the control negative group, no cecal lesions were observed, and the cecum appeared normal. However, in the control infected untreated group, there was severe enlargement of the cecum, with a bloody cecal core, and the cecum weight with its content recorded approximately 40 g. These changes were also present, though less severe, in the YS and YSN groups across both trials at the first three days, but the lesion score improved more rapid than the infected untreated group. Microscopically, the number of Eimerian developmental stages in the cecal wall of the YS-treated groups was significantly reduced compared to the endogenous stages in the infected untreated group (Fig. 3C & D). This reduction was associated with pathological alterations of the mucosal and submucosal surfaces, showing a marked decrease in the number of Eimerian developmental stages within the infected epithelium. Additionally, oocysts were observed amidst lymphocytic infiltration and macrophages at day 9 post-infection (Fig 3 E&F).

6. Biochemical analysis

All tested parameters of creatinine, blood urea nitrogen (BUN), GPT and GOT were of no significant difference in control negative and the infected treated groups at day 12 post infection (Table 5). It was noticed that all trial groups showed BUN within the normal range (Table 5).

Table 4: Carcass meat cuts of the carcasses in all groups on day 35 at the end of the experiment

group	Negative control	infected untreated	Y. schidigera 5% treatment	Y. schidigera nanoemulsion 5% treatment	Y. shidegra 5% prophylaxis	Nano Y. schidigera nanoemulsion 5% prophylaxis
Life bwt	2033 ± 156	1527 ± 72.5	1805 ± 124	1712 ± 103*	1823 ± 0.00	1850 ± 157
DE feathered carcass	1793 ± 129	1257 ± 42.5	1470 ± 96.4*	1360 ± 52.9*	1473 ± 377*	1499 ± 98.0*
Whole chicken without head and neck	1540 ± 147	981 ± 74.9	1229 ± 121*	1140 ± 47.7*	1247 ± 342*	1246 ± 77.3*
Breast quarter	425 ± 60	265 ± 10.7	361 ± 48.9	327 ± 20.0*	367 ± 89.3	357 ± 19.7*
Split breast	326 ± 46	200 ± 23.9	257 ± 52.7*	225 ± 9.29*	264 ± 65.7*	251 ± 18.3*
Leg quarter	311 ± 14.4	204 ± 24.8	244 ± 13.7*	249 ± 21.8*	247 ± 77.4*	255 ± 20.8*
Liver	46.7 ± 0.6	42.7 ± 4.16	41.7 ± 5.68	44.3 ± 5.51	41.0 ± 4.36	46.3 ± 1.53
Heart	9.33 ± 1.04	8.27 ± 0.68	9.33 ± 2.31	7.67 ± 1.15	8.00 ± 2.65	9.67 ± 0.58
Spleen	2.93 ± 0.20	2.23 ± 0.15	2.71 ± 0.61	2.77 ± 1.27	3.01 ± 0.01	2.06 ± 0.06
Gizzard & proventriculus	42.3 ± 7.37	41.3 ± 1.53	38.7 ± 7.23	37.0 ± 3.00	39.7 ± 6.66	41.3 ± 2.08
Whole intestine	126 ± 6.08	148 ± 26.7	120 ± 25.1	112 ± 7.23	115 ± 14.1	132 ± 22.5
Caecum	16.8 ± 0.58	10.3 ± 2.25	11.0 ± 2.65*	9.67 ± 1.53*	11.7 ± 1.53	14.7 ± 7.02

(*) significant for negative control

Table 5: Blood chemistry of different groups at the end of the experiment

Groups	Creatinin	BUN	ALT	AST
Negative control, uninfected untreated	2.48 ± 0.15	2.86 ± 0.15	26.3 ± 2.31	183 ± 5.57
Positive control, infected untreated)	1.91 ± 0.24	3.48 ± 0.18	27.3 ± 3.21	179 ± 7.23
Yucca shidegra extract 5% (YS) treatment	2.13 ± 0.17	3.68 ± 0.25*	28.1 ± 3.96	163 ± 14.6
Yucca shidegra extract 5% nanoemulsion treatment	2.03 ± 0.13	3.78 ± 0.29*	26.5 ± 8.05	163 ± 7.64
Yucca shidegra extract 5% prophylaxis	2.42 ± 0.18	3.80 ± 0.13*	28.7 ± 6.51	156 ± 11.2
Yucca schidigera extract 5% nanoemulsion prophylaxis	2.26 ± 0.43	3.61 ± 0.41*	27.0 ± 2.00	156 ± 16.5

(*) significant for negative control.
- BUN = blood urea nitrogen, Alanine Aminotransferase (ALT). Aspartate Aminotransferase (AST).

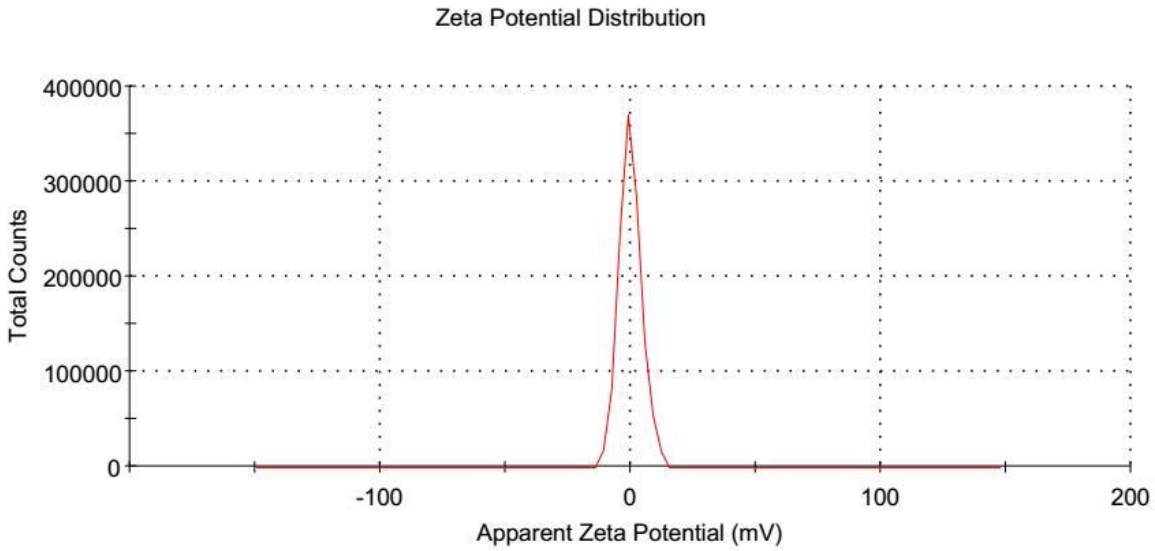

Figure 1: Size distribution of *Youka shidigra* extract nanoemulsion by intensity using Zeta apparatus

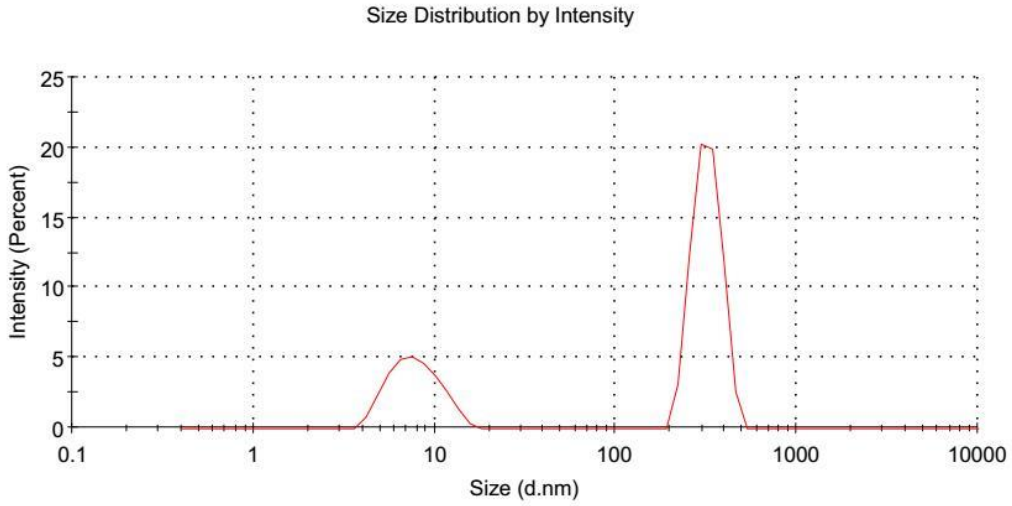

Figure 2: Zeta potential distribution of *Youka shidigra* extract nanoemulsion by Zeta apparatus.

7. Hematological parameters

At day 6 post-infection, all infected groups of chicks revealed sharply declined in RBCs and Hb compared to the control negative birds (Table 6). Thus, all infected groups suffered from severe anemia due to the loss of blood in the diarrhea, with these groups revealing RBC counts lower than one million. WBC counts showed a significantly higher level than the control negative group that's due to infection and cecal tissue inflammation (Table 6). At day 9 post-infection, RBCs and Hb were restored and began to increase but remained lower than the control negative birds, while WBCs were still higher than the control negative group (Table 7). Moreover, platelet counts increased significantly higher than the control negative chicks (Table 7). At day 12 post-infection, the whole parameters of RBCs, the signs of anemia disappeared with nearly normal levels of RBCs, Hb, Hct, MCV, MCH and MCHC (Table 8). Additionally, platelet counts were still significant higher than the control negative chicks (Table 8).

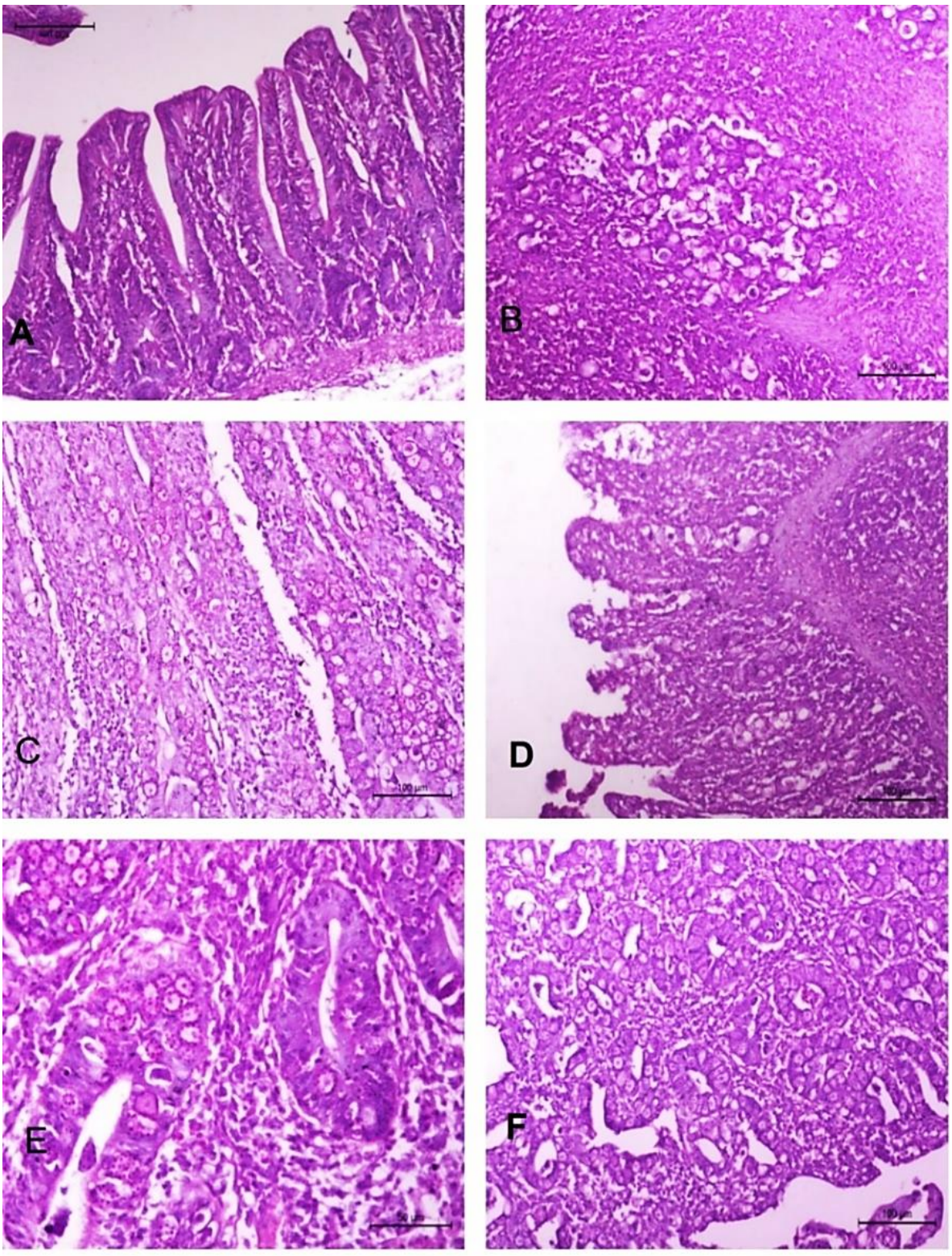

Fig. 3: Histopathological pictures: A. Negative control uninfected untreated showed normal intestinal villi, B. Positive control infected untreated group showed cecal mucosa severely congested with some coagulative necrosis, epithelium contains macrogametocytes and microgametocytes with eosinophilic infilt-ration of mononuclear cells. C. YS treatment, D. YSN treatment, observed different stages of oocysts, micro and macrogametocytes. E. YS prophylaxis F. YSN prophylaxis, the cecal mucosa congested and the epithelial cells contained macrogametocytes and micro-gametocytes with eosinophilic cells.

Table 6: Haematological pictures at day 6 post infection in all groups

group	Negative control	infected untreated	Yucca schidigera 5 % treatment	Nano Yucca schidigera 5 % treatment	Yucka shidegra 5 % prophylaxis	Nano Yucca schidigera 5 % prophylaxis
Wbcs	36.3 ± 5.68	59.0 ± 4.36	52.7 ± 8.02*	54.7 ± 6.51*	58.0 ± 6.55*	54.4 ± 6.07*
Lym	30.0 ± 2.00	30.0 ± 1.00	43.2 ± 0.76*	41.3 ± 2.52*	33.0 ± 8.18	36.0 ± 6.14
Mid	11.7 ± 2.08	23.0 ± 1.00	15.0 ± 1.00	15.3 ± 1.53	14.7 ± 3.06	14.0 ± 2.65
Gran	58.3 ± 4.04	47.0 ± 1.00	41.8 ± 1.75*	43.3 ± 2.08*	51.6 ± 7.89	49.9 ± 5.05
RBCs	2.43 ± 0.02	0.81 ± 0.01	0.83 ± 0.02*	0.84 ± 0.03*	0.84 ± 0.04*	0.85 ± 0.02*
Hgb	10.2 ± 0.26	3.68 ± 0.03	3.82 ± 0.07*	3.72 ± 0.13*	3.82 ± 0.08*	3.72 ± 0.08*
Hct	25.9 ± 0.12	9.28 ± 0.20	9.70 ± 0.14*	9.67 ± 0.32*	9.95 ± 0.13*	9.71 ± 0.19*
MCV	107 ± 0.55	113 ± 2.46	117 ± 4.40*	115 ± 7.92*	119 ± 3.47*	114 ± 3.29
MCH	42.0 ± 0.87	45.7 ± 0.57	46.1 ± 1.49*	43.6 ± 3.00	45.5 ± 1.32*	43.6 ± 1.46
MCHC	39.3 ± 0.91	39.7 ± 0.88	39.4 ± 0.63	38.5 ± 1.54	38.4 ± 0.41	38.3 ± 1.45
Platelets	2.92 ± 0.27	3.29 ± 0.31	2.62 ± 0.49	2.44 ± 0.33	3.62 ± 0.58*	5.78 ± 0.21

(*) significant for negative control

Table 7: Haematological pictures at day 9 post infection in all groups

group	Negative control	infected untreated	Yucca schidigera 5 % treatment	Nano Yucca schidigera 5 % treatment	Yucka shidegra 5 % prophylaxis	Nano Yucca schidigera 5 % prophylaxis
WBCs	63.0 ± 3.00	103 ± 15.3	76.4 ± 9.16	68.5 ± 4.13	74.6 ± 9.08	97.4± 14.6*
Lym	28.0 ± 5.89	18.2 ± 6.41	36.2± 5.16*	34.3 ± 3.85	31.5 ± 6.19	23.2 ± 7.82
Mid	9.83 ± 1.59	13.2 ± 4.19	12.3 ± 1.01	11.6 ± 0.71	10.9 ± 1.59	9.83 ± 2.11
Gran	62.1 ± 7.58	68.6 ± 2.31	51.5 ± 6.13	54.1 ± 5.55	57.5 ± 7.78	66.9 ± 9.24
RBCs	2.58 ± 0.14	1.53 ± 0.15	2.14± 0.11*	2.33 ± 0.47	2.40 ± 0.02	1.95± 0.23*
Hb	11.2 ± 0.57	8.00 ± 0.26	8.63± 0.38*	9.30± 1.65*	9.73± 0.42*	8.00± 0.78*
Hct	29.7 ± 1.53	20.2 ± 2.47	24.9 ± 0.25	25.9 ± 5.11	26.3 ± 0.56	22.6 ± 0.75
MCV	112 ± 1.73	126 ± 7.51	117 ± 7.17	112 ± 3.72*	110 ± 1.77	117 ± 10.7
MCH	44.7 ± 2.08	52.7 ± 7.51	40.2 ± 0.55	40.1 ± 1.45	40.5 ± 1.57	41.1 ± 0.98
MCHC	39.2 ± 1.57	41.2 ± 5.08	34.6 ± 1.85	35.9 ± 0.90	36.9 ± 1.72	35.4 ± 2.32
Platelets	3.30 ± 0.62	44.3 ± 1.15	49.0 ±5.19*	42.0 ± 4.58*	40.7 ± 3.06*	46.0 ± 4.58*

(*) significant for negative control

Table 8: Hematological pictures at day 12 post infection in all groups

Group	Negative control	infected untreated	Yucca schidigera 5 % treatment	Nano Yucca schidigera 5 % treatment	Yucka shidegra 5 % prophylaxis	Nano Yucca schidigera 5 % prophylaxis
WBCs	65.3 ± 2.52	69.9 ± 5.66	90.5± 16.9*	70.7 ± 4.12	78.9 ± 0.45	74.1 ± 7.59
Lym	30.4 ± 5.90	31.3 ± 1.78	27.9 ± 7.25	31.2 ± 0.42	30.1 ± 0.78	32.0 ± 3.46
Mid	10.7 ± 0.70	10.6 ± 0.31	12.4 ± 2.75	11.8 ± 0.92	10.9 ± 0.76	11.5 ± 0.60
Gran	58.9 ± 6.59	58.1 ± 2.08	59.6 ± 4.50	56.9 ± 0.90	58.0 ± 2.05	56.4 ± 4.07
RBCs	2.60 ± 0.26	2.59 ± 0.10	2.76 ± 0.03	2.63 ± 0.09	2.74 ± 0.12	2.79 ± 0.31
Hb	10.7 ± 1.09	9.73 ± 0.57	9.77 ± 0.12	9.53 ± 0.64	9.97 ± 0.41	9.83 ± 1.21
Hct	28.9 ± 3.23	27.6 ± 2.51	30.3 ± 2.17	28.3 ± 2.58	28.7 ± 1.20	28.4 ± 3.26
MCV	114 ± 6.13	109 ± 8.50	110 ± 6.83	108 ± 10.0	108 ± 3.11	102 ± 1.25
MCH	41.8 ± 2.01	39.3 ± 1.53	35.3 ± 0.21	36.2 ± 2.10	37.6 ± 2.00	35.1 ± 0.55
MCHC	38.8 ± 1.01	37.6 ± 0.97	32.2 ± 2.03	33.7 ± 1.24	34.7 ± 1.17	34.6 ± 0.31
Platelets	2.77 ± 0.15	29.7 ± 14.6	23.3± 4.04*	21.3 ±2.08*	17.3± 4.04*	21.7± 0.58*

(*) significant for negative control

DISCUSSION

In this study, the results showed that the plant extracts helped in regaining body weight and vitality significantly better than the control infected untreated group.

When YS extract, both in its normal form and as a nanoemulsion, was used in phytotherapy to treat *E. tenella* infection in chicks, there was an increase in weight gain and a reduction in oocyst numbers. No significant difference was observed between the YS and YSN forms in their effectiveness as treatments or as prophylactic measures against the infection. Additionally, YS and YSN treatments resulted in a reduction in daily oocyst shedding. Despite the infection, YS and YSN helped preserve body weight and improved carcass meat cuts quality. The YS extract used contains 35% saponins. Hashemi *et al.* (2008) reported that plant extracts protect the intestinal wall from damage caused by coccidial multiplication. Thus, the current results of maintaining growth and body weight resemble the findings of Youssef *et al.* (2020) and Bafundo *et al.* (2020), who found that a combination of quillaja and yucca saponins for broilers reduced oocyst count and improved body weight. Additionally, Xiangbing Mao *et al.* (2023) reported that dietary supplementation of *Yucca shidigera* extract improved digestion, absorption, and promoted growth with a reduction of the negative effects of coccidian infection. Furthermore, Chen and Yu (2020) suggested that the mitigation of *Yucca shidigera* to the coccidian negative effect is due to gut barrier function improvement. Augustin *et al.* (2011) and Fleck *et al.* (2018) suggested that quillaja and yucca saponins can alter the integrity of biological membranes, cellular permeability, and membrane porosity. Moreover, Alagawany *et al.* (2016) mentioned that *Yucca shidigera* supplement improved barrier function in the broilers proximal intestine. Additionally, Kozłowski *et al.* (2022) found that YS decreased oocyst count and improved the body performance of the treated chickens. It was noticed that the treatment may exacerbate the infection. In the crypt, fully mature gamonts are present in nearly every single cell. The so-called crowding effect can result from being heavily infected. Overcrowding of cells with developmental stages can cause the crowding effect, which will diminish oocyst shedding and the production of subsequent stages (Williams 2001). This could be the primary reason why this group's shedding has decreased. The similar thing treated group: the extract or nanoemulsion did not appear to directly kill or alter the parasitic stages. The decrease in oocyst shedding and the similarity in lesion scores between the infected and treated groups could possibly be explained by the crowding effect (Williams 2001). The findings of improved body weight of treated chicks by YS were supported by Sahoo *et al.* (2015), Sun *et al.* (2017), and Khaskheli *et al.* (2020).

Both forms of YS and YSN were safe to the liver and kidney, and that the liver function and kidney function tests recorded normal levels at the end of the experiment, similar to previous works (Mondal *et al.,* 2011; Adamu *et al.,* 2013; Alagawany *et al.,* 2015; Reis *et al.,* 2018). Even the numbers of BUN are variable, but still within the normal values. Where the average level of blood urea nitrogen in native chickens was overall 5.31±1.36 with a range of 4.40±0.68 -6.38±1.42 (Ismoyowati *et al.,* 2022).

In our study, the haematological pictures were adversely affected directly postinfection, and the infected chicks in all groups suffered from severe anemia. This is due to the formation of schizonts of Eimeria in the intestinal epithelium, resulting in rupturing cells with blood loss and bloody diarrhea (Nayak, 1985; Ellakany *et al.,* 2011). On day 12, post-

infection, the hemograms of the infected birds were restored to normal levels. This finding agreed with Mondal *et al.* (2011) and Li *et al.* (2020), who reported that essential oils reduced the damage during coccidiosis and reduced blood loss, resulting in improved hematological profiles in broiler chicks.

Coccidiosis in chickens may lead to a reduction in breast meat (Rajput *et al.*, 2013; Shaw *et al.*, 2012). Interestingly, the YS and YSN groups had meat cuts better than the infected untreated group. This was supported by the findings of Zhang *et al.* (2015) and Partovi *et al.* (2019), who found that curcumin and its nanoform improved breast meat in the treated broilers. This finding may be related to an increase in blood loss and a decrease in mineral absorption in muscle tissue in the infected bird (Anosa and Okoro, 2011). On the contrary, Olschlager *et al.* (2019) showed that *Yucca shidigera* has no significant effect on performance-treated chickens.

In the current investigation, the nanoemulsion version of YS produced non-significant findings similar to the conventional form. The effective encapsulation, preservation, and distribution of sensitive bioactive components can be achieved through the development of nanoscale biocompatible systems (Demisli *et al.*, 2020). Among these nanoscale systems, nanoemulsions made of several natural oils and a biological amphiphilic molecule have been studied as encapsulating media for bioactive substances that may be used in food and medicine (Tayeb *et al.*, 2021).

In conclusion, YS and its nanoemulsion form reduce the negative effects of cecal coccidiosis by reducing the number of oocysts, promoting quick recovery from the disease, and improving body weight. Even so, this extract alone cannot control the infection.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used [https://chatgpt.com/] to improve the readability of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and took(s) full responsibility for the content of the publication.

Competing interests statement: "The authors declare that they have no competing interests".

Ethics statement

The experiments were carried out in compliance with the protocols and ethical standards set out by Beni Suef University's Faculty of Veterinary Medicine in Egypt (2017-BSUV-11).

Author contributions

Conceptualization; SMA, AAE; Data curation; ENE,; Formal analysis; HEH, SMA; Funding acquisition; AAE; Investigation; HEH; Methodology; HEH, ENE; Supervision; AAE, SMA; Validation; SMA; Visualization; HEH, ENE; Roles/Writing - original draft; HEH, ENE; Writing - review & editing; AAE, SMA.

Funding: not applicable

Acknowledgements

The authors appreciate the help of Dr. Samar M Ibrahium for help in statistical analysis.

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كفاءة مستحلب النانو لمستخلص اليوكا شيديجيرا فى السيطرة على الإصابة بالأيميريا تينيلا فى دجاج التسمين

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تشير أدلة علمية إلى أن تكنولوجيا النانو قد تزيد من استقرار وتوصيل وامتصاص الأدوية على مستوى الخلايا. لذلك، تم تصميم هذه الدراسة لمعرفة فعالية مستحلب النانو لمستخلص نبات يوكا شيدجيرا في الوقاية من مرض الكوكسيديا وعلاجه لدى دجاج التسمين. تم تحضير مستخلص يوكا شيدجيرا بنسبة ٥٪ كمستحلب نانوي ، وتم توصيفه باستخدام جهاز قياس حجم الجسيمات و جهاز زيتا المحتمل. تم تقسيم الكتاكيت عمر يوم واحد الى ست مجموعات كل منها ٣٥ كتكوتا، مجموعتان ضابطتان سلبية وإيجابية (مصابة بدون علاج)، ومجموعتين للعلاج باستخدام المستخلص أ و مستحلب النانو، ومجموعتين للوقاية باستخدام المستخلص أ و مستحلب النانو ، تم إعطاء مجموعات العلاج المستخلص و مستحلب النانو في اليوم الرابع بعد الإصابة، بينما تلقت مجموعات الوقاية المستخلص و مستحلب النانو قبل ٥ أيام من الإصابة. كانت جرعات المستخلص ومستحلب النانو ١٠٠ مجم/ لتر في الماء. تم إصابة جميع المجموعات باستثناء مجموعة الضابطة السلبية عن طريق الفم بحوالي ٢٥ × 10^3 من الأكياس المتحوصلة لطفيلي *إيميريا تينيلا* لكل فرخ في اليوم ٢٣ من العمر. أظهرت كل من مجموعات العلاج والوقاية انخفاضًا كبيرًا في عدد الأكياس البيضية، وتعافيًا سريعًا من المرض، وزيادة أفضل في الوزن مقارنة بالمجموعة المصابة غير المعالجة. أظهرت الفحوصات الكيميائية الدموية وصور الدم الكاملة في مجموعات العلاج والوقاية عدم وجود فروق ذات دلالة إحصائية عن مجموعة التحكم المصابة غير المعالجة. أظهرت الصور المرضية عددًا أقل من المراحل الطفيلية في نسيج الأعور في المجموعات المعالجة مقارنة بالمجموعة المصابة غير المعالجة. الخلاصة، يقلل كل من المستخلص ومستحلب النانو من تأثيرات الطفيلي إيميريا تينيلا من خلال تقليل عدد الأكياس البيضية، وتحسين زيادة الوزن. ولم تُظهر الدراسة فرقًا كبيرًا بين المستخلص وشكل مستحلب النانو الخاص به.

الكلمات المفتاحية: كتاكيت – مستحلب النانو – يوكا شيديجيرا – ايميريا تينيلا – وزن الجسم – قياسات بيوكيميائية