The pharmacological impact of *Astragalus membranaceus* against coccidial and bacterial infection *in vitro*

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Background
Astragalus polysaccharides (APS) are a novel macromolecule extracted from the herbal plant *Astragali radix* with potential biological activity such as antioxidant, anti-inflammatory, antidiabetic, anticancer, and immunomodulatory properties.
Objectives
The present research emphasizes on some of the biological characteristics of this product including its phytochemical screening, its effective LD_{50}, its antioxidant, anti-inflammatory, anticoccidial, and antimicrobial activities *in vitro*.
Materials and methods
Phytochemical screening of the tested extract proved that it contained alkaloids, flavonoids, and glycoside components. Testing its efficacy as bactericidal versus *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Pasteurella multocida* and *Staphylococcus aureus* its value as a coccidiocidal drug against five chicken *Eimeria* species oocysts and its effect on the level of DNA genotoxic damage using comet assay proved high significant efficacy ($P \leq 0.05$) in the form of marked inhibition zone of bacteria, considerable sporulation inhibition percentage in oocysts as well as high genotoxic damages in the DNA.
Result and conclusion
The study proved the presence of a direct relationship between the increase in APS concentrations and exposure time and the rate of sporulation inhibition and DNA damage in oocysts subjected to various doses of APS. This DNA damage was determined by marked variations in tail's length (μm), the percentage of DNA in the tail segment, and tail's moment were used to demonstrate this relationship (μm). In conclusion, APS proved to be a potential herbal to have anticoccidial and antibacterial attributes in controlling both infections in chickens.

Keywords:
antibacterial activity, anticoccidial, *Astragalus membranaceus*, comet assay, poultry

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Introduction

The serious parasitic condition known as 'avian coccidiosis' in chickens is brought on by the intracellular apicomplexan protozoan *Eimeria* species. This deadly condition affects the gastrointestinal tract [1]. The intracellular and extracellular stages of the life cycle of the *Eimeria* species induce a significant inflammatory response that affects tissue oxidative stress and lipid peroxidation damage, diarrheal bleeding, poor growth, increased susceptibility to other disease agents, and, in severe cases, fatality [2]. Numerous anticoccidial drugs and immunization have been used to treat and prevent this illness; however, there have been some negative effects detected [3]. Customers' concerns about drug residues, the drawbacks of conventional vaccines, the toxicity of ionophores to poultry, and the resistance of some *Eimeria* spp. to some ionophores have all led scientists to concentrate their research on creating safe, effective alternatives using natural ingredients [4,5].

One of these safe substitutes for anticoccidial medications that comply with the 'anticoccidial chemical-free' standards is herbal medicine. The presence of anticoccidial properties in natural herbal treatments (or their extracts) has been reported frequently [6]. The effect of herbal additives on avian coccidiosis is based on decreasing the number of oocysts because phenolic compounds in herbal extracts react with cytoplasmic membranes to cause coccidial cell death; increasing the level of intestinal lipid peroxidation, facilitating the repair of epithelial damage, and increasing the epithelial turnover to

decrease intestinal permeability brought on by *Eimeria* spp. [7].

Antibiotics frequently lose their efficacy over time due to the emergence and spread of drug resistance in bacterial infections, creating an apparent 'antibiotic resistance crisis,' and immune-mediated diseases brought on by bacterial drug resistance significantly increase annual medical costs, amounting to billions of dollars. There is an urgent need for 'novel antimicrobial medications and treatment techniques as the problem's severity increases [8].

More than 2000 years ago, *Astragali radix*, a widely used herbal remedy, was first made from the roots of *Astragalus membranaceus*. *A. radix* is currently used to heal wounds, uterine prolapse, anemia, and fever. Around 200 different substances, including isoflavonoids, amino acids, saponins, and polysaccharides, are found in *A. radix* [9,10].

A significant component of the *A.* radix, *Astragalus* polysaccharides (APS) may have biological effects that include anti-inflammatory, antioxidant, antidiabetic, anticancer, and immunomodulatory properties [11,12]. To present experimental research foundations and clinical application data for the clinical use of APS, this study focuses on the most recent breakthroughs in research on APS therapeutic pathways and pharmaceutics.

The effectiveness of APS against five *Eimeria* species that infect poultry was investigated. In addition, the inhibitory effects on developing oocysts were assessed. The *in vitro* investigation includes antioxidant, anticoccidial, and antibacterial activities [minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)] of this extract, which are being researched. To find resin, alkaloids, tannins, saponins, flavonoids, and glycosides, phytochemicals were screened. Using the 1, 1-diphenyl-2-picryl hydrazyl (DPPH) technique, the antioxidant pattern was examined. Finally, the comet assay was done to assess the level of DNA genotoxic damage in the subjected and control oocysts.

Materials and methods

Extraction of *Astragalus* polysaccharides

The root of the *A. membranaceus* plant was used for extraction of the target materials (dried root of superior quality from Qi Jing Ltd, Beijing, China). APS extraction was done in the Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt. Sigma Aldrich Company, Merck Ltd., Burlington, Massachusetts, United States was used to obtain ethanol, CaO, and Na_2CO_3. According to Zhu *et al.* [13], a crude water extract of APS was evaporated to a predetermined volume, 100% ethanol was added, the combination was allowed to sit overnight, and then the mixture was precipitated and centrifuged to separate the extract.

Ethical approval: the Faculty of Veterinary Medicine and the Institutional Animal Care and Use Ethical Committee of Cairo University Ethics Committee, Giza, Egypt (Vet CU 01122022597) demonstrated that all steps of the protocol were followed.

Phytochemical screening

Astragalus polysaccharides extract preliminary phytochemical testing

Using standard phytochemical methods, alkaloids, tannins, flavonoids, carbohydrate/glycosides, and resin were all found, according to Pant *et al.* [14].

Total phenolic content determination

The Folin-Ciocalteu technique previously described [15] was determined spectrophotometrically. A measure of 200 µl of APS was diluted to a concentration of 1 mg/ml in 3 ml of distilled water, forcefully mixed for 3 min with 0.5 ml of Folin-Ciocalteu reagent, and then 2 ml of 20% (w/v) sodium carbonate was added. A 650 nm absorbance was measured on the combination after an additional 60 min of total darkness. Using the calibration curve, the total phenolic content was calculated and represented as micrograms (g) of gallic acid equivalent per gram of dry APS. The regression equation for the standard plot was used to calculate the total phenolic content (y=105.51x +2.1824, R^2=0.9978).

Calculating the content of total flavonoids

The aluminum chloride colorimetric method is used to assess the concentration of total flavonoids [15]. After spending 30 min at room temperature, 1 ml of the sample was combined with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. At 420 nm, the absorbance was determined. Rutin (1 mg/ml) was utilized as the reference. The regression equation for the standard plot was used to calculate the flavonoid content.

Antioxidant activity 1, 1-diphenyl-2-picryl hydrazyl radical scavenging method

Free radical scavenging activity of different extracts of the plant was measured by DPPH [16]. In brief, a

0.1 mM DPPH solution in ethanol was prepared. This solution (1 ml) was mixed with 3 ml of various ethanol extracts at various concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19 µg/ml). Only extracts that are soluble in ethanol are used in this study, and their various concentrations were prepared using the dilution method. The mixture was vigorously shaken and allowed to stand at room temperature for 30 min. The absorbance was then measured at 517 nm with a spectrophotometer (ultraviolet-visible Milton Roy). The reference standard compound used was ascorbic acid, and the experiment was carried out in triplicate. The IC_{50} value of the sample, which is the concentration of the sample required to inhibit 50% of the DPPH free radical, was calculated using the log-dose inhibition curve. The higher free radical activity was indicated by the lower absorbance of the reaction mixture. The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) or percent inhibition= A_0-A_1/A_0×100,

where A_0 represented the absorbance of the control reaction and A_1 represented the absorbance in the presence of the test or standard sample.

Determination of LD_{50} of Astragalus polysaccharides

It was determined in the manner given by Randhawa [17]. Five groups of five mice, each weighing 20–25 g, were used to achieve this. Mice were administered dosages of the examined medication ranging from 1 to 5 g/kg body weight for APS. Each group's toxic symptoms, mortality rate, and postmortem results were noted within 24 h of injection. The drug's LD_{50} was estimated using the formula below:

LD_{50}=DM-($\sum A \times B$)/N.

[DM: the highest dose that is lethal to all animals, A: the average number of dead animals between two successive groups, B: the constant factor between two subsequent doses, N: the number of animals in each group, Σ: the sum of (A×B)].

Determination of anti-inflammatory activity (in vitro COX-1/ COX-2 inhibition)

The capability of the test APS to inhibit both COX-1 and COX-2 was assessed using a COX inhibitor screening assay kit (Item No. 560131; Cayman Chemicals, Ann Arbor, Michigan, USA), which directly evaluates PGF2 through Sncl2 reduction of COX-derived PGH2 generated in the COX reaction. A broad antiserum that binds to all of the essential PG components is used in an enzyme immunoassay to measure the prostanoid product [18].

This assay contains recombinant COX-2 enzymes from both humans and ovine, enabling the user to search for inhibitors that target a particular isozyme. This test is based on the competition for a limited supply of PG antiserum between PGs and a PG-acetylcholinesterase conjugate (PG tracer). As the PG tracer concentration remains constant while the PG concentration varies, the amount of PG tracer that can bind to the PG antiserum is inversely proportional to the PG concentration in the well. In the well that was previously attached to it, this mouse monoclonal anti-rabbit antibody binds to the rabbit antiserum-PG complex (either free or tracer).

The plate is rinsed to remove any reagents that are not bound to the plate before adding Ellman's reagent (which includes the acetylcholinesterase substrate) to the well. The unique yellow color produced by this enzyme activity absorbs strongly at 412 nm. The ratio of (bonded PG tracer)/(PG) absorbance, which measures the quantity of PG tracer bound to the well and the amount of free PG that was present in the well during incubation, determines the intensity of this coloring.

Oocysts of Eimeria species

Eimeria spp. oocysts were separated from the ceca of recently slaughtered naturally infected chickens in the Faculty of Veterinary Medicine at Cairo University's Parasitology Department [5]. Oocysts were cleansed by washing multiple times and precipitation in water after the cecal contents were gathered, blended, and sieved to eliminate the coarse fecal debris. The oocysts were separated using the concentration flotation method, identified as previously described [1], and then stored in potassium dichromate (2.5%) until use. The following five *Eimeria* species' oocysts were found and employed in the current study*: E. tenella* (69%), *E. maxima* (10%), *E. acervulina* (9%), *E. necatrix* (8%), and *E. mitis* (4%).

Investigating Astragalus polysaccharides coccidiocidal properties in vitro

On *Eimeria* spp., the generated APS's anticoccidial properties were evaluated: Sporulated and nonsporulated oocysts. Of the *Eimeria* spp., which had just been washed and gathered, in this experiment, three doses were evaluated (10, 25, and 50 mg)/5 ml potassium dichromate containing 1×10^4

oocysts [19]. Unsporulated oocysts were distributed as a thin layer in the bottom of 5 cm diameter Petri dishes for a series of increasing concentrations of APS. Oocysts in triplicates at 25–29°C and 80% relative humidity served as the control. They were subjected to 1, 3, 6, 9, 12, 24, and 36 h. As a reference medication, Toltrazuril (2.5%) (Toltacoccin 2.5%) was used concurrently at a concentration of 25 ppm [20]. Furthermore, control oocysts in pure water were correlated. The oocysts were repeatedly washed and sedimented once the exposure period was over, and a testing solution was removed. To induce sporulation, the subjected immature oocysts were incubated in potassium dichromate for 5–7 days at 28°C.

The McMaster approach was used to count sporulated and nonsporulated oocysts in order to assess the coccidiocidal efficacy of APS on the subjected *Eimeria* spp. unsporulated oocysts [5]. The percentage of sporulation inhibition was then calculated from a total of 100 oocysts (three replicates) by counting the quantity of unsporulated oocysts. Using the following equation determine the percentage of sporulation inhibition:

$$\text{Sporulation inhibition}\% = \text{Sp}\%\text{of control}\text{Sp}\% \text{ of extract}/\text{Sp}\%\text{of control} \times 100$$

Single-cell gel electrophoresis comet assay was used to assess how the products influenced the DNA damage in the subjected oocysts [21]. A sufficient quantity of the immature oocysts from the naturally infected chickens was collected, sporulated in Petri dishes using a 2.5% potassium dichromate solution, and then cultured at 28°C for 5–7 days. Nevertheless, a study examined the coccidiocidal impact of APS on sporulated oocysts from *Eimeria* spp. Following removal of potassium dichromate, the calculated LC_{50} and LC_{90} of APS that were previously recorded after exposure of the immature oocysts were subjected to the modified number of sporulated oocysts counted using the McMaster approach. The product's effects on the DNA damage in the subjected oocysts were subsequently evaluated using the comet assay [21].

Bacterial strains

Escherichia coli (ATTC 25922), *Salmonella typhimurium* (ATTC 13311), *Klebsiella pneumoniae* (ATTC 10031), *Pasteurella multocida* (ATTC 43137), and *Staphylococcus aureus* (ATTC 25923) were cultured from the ceca and intestine of chicken at the Microbiology Department, Faculty of Veterinary Medicine, Cairo University according to Raheel *et al.* [22].

Determination of the minimum inhibitory concentration and the minimum bactericidal concentration

Preparation of the bacterial suspension

The purpose was to obtain single pure colonies through quadrant streaking of the tested micro-organism on Mueller-Hinton agar (MHA) followed by 18–24 h at 37°C incubation. The required suspension can be prepared by two different techniques which are the colony suspension method and the growth method [23].

(1) Colony suspension method: select three to five similar fresh colonies from the MHA plates that are previously prepared. A sterile loop or cotton swab will be used to touch the center of each chosen colony before it is inoculated into a sealed sterile saline tube. Use a vortex to thoroughly blend the suspension.
(2) Growth method: pick three to five comparable new colonies from the MHA plates that have already been made. Each chosen colony will be touched in the center with a sterile loop or cotton swab before being injected into a sterile Mueller-Hinton broth (MHB) tube. To appropriately mix the suspension, create a vortex. Continue to shake the incubator at 37°C and 225 rpm once the inoculated soup becomes visibly turbid.

The 0.5 McFarland Standard was then used to compare the obtained turbidity. A white background, distinct black lines, and decent lighting are helpful for comparison. Add sterile distilled water, saline, or broth to the suspension if it has an excessive amount of turbidity. The bacterial culture should continue to be incubated if the suspension's turbidity is too low.

Antimicrobial testing: prepare 10-fold tested APS serial dilutions in sterile MHB test tubes. Completely combine using a vortex mix. Fill a test tube with 1 ml of each APS dilution for each isolate being examined. One milliliter of sterile soup free of antibacterial agents should be added to the control tubes. By adding 200 ml of the bacterial suspension to 19.8 ml of sterile MHB in a sterile 50 ml Erlenmeyer flask, the bacterial suspension is diluted by a factor of 100 to yield a 20 ml full inoculum size. One milliliter of the bacterial stock suspension should be added to each test tube containing 1 ml of each APS concentration and one control test tube. According toAhmed *et al.* [24], 100 µl of each dilution was added to MHA plates, and the plates were incubated at 37°C for 16–20 h.

Following incubation, the MIC and MBC can be defined as follows: MIC is the last dilution that shows visible turbidity in the MHB tube and the fewest visible colonies on the MHA plate, whereas MBC is the first dilution that shows no visible turbidity in the MHB tube and no visible colony growth on the MHA plate.

The comet assay's assessment of DNA damage

The comet assay was used to assess DNA damage [21]. DNA damage was observed in subjected and controlled *Eimeria* spp. oocysts using a fluorescent microscope with a ×40 objective and the image analysis program Komet 5. (Kinetic Imaging Ltd, Liverpool, UK). By calculating the length and percentage of DNA movement, Komet 5 can assess the quantitative and qualitative level of DNA damage in cells. The software then computes the tail moment. The percentage of DNA in the tail (% DNA tail) was used to assess DNA damage. Hundred cells were chosen at random and examined for each treatment. The formula was used to compute the comet assay score in the 0–400 arbitrary unit range [21].

Statistical analysis

Analysis of variance was applied to statistically analyze the data collected, and the least significant difference at *P* value less than or equal to 0.05 was used to compare groups [25]. They were computerized using IBM SPSS Statistics 27.

Results and discussion

Nowadays, natural medicines are applied to treat diseases. Synthetic medications, however, are risky and have severe adverse effects that compromise public health. Nevertheless, regular use of synthetic medications might result in major problems such as the emergence of drug resistance. Herbal treatments are regaining popularity because they have few adverse effects [26,27]. Medicinal plants and prospective herbs, particularly those with bioactive properties such as antibacterial, antioxidant, antidiabetics, antiparasitic, and anticancerous, are still being widely subjected for improvement in the field of human, animal, and poultry health [28].

The chemical structure of *A. membranaceus* was previously reported [29] and demonstrated it involved polysaccharides, saponins, and flavonoids, which are the most biologically active constituents, along with sucrose, phenolic acids, and amino acids. The principal bioactive constituents essential for the medicinal actions of AM's desiccated roots are flavonoids, polysaccharides, and the saponins astragalosides, mainly polysaccharides. *Astragalus* (As) I, II, and III are among the polysaccharides found in AM. As-I is a glucose-based and arabinose-based carbohydrate chain, whereas As-II is a glucose-based, arabinose-based, and rhamnose-based carbohydrate chain. D-glucose is the only component of As-III. The most predominant polysaccharide in AM is astragalin [30]. In addition to isoastragalosides, I–III, astragalosides are steroidal saponins that are found in AM and have been classified into seven groups based on the cycloastragenol structure [31]. Flavonoids (flavones, flavolins, flavonols, and isoflavones) are present in AM in free or glycosidic forms [32,33].

The power of herbal medicine has a great role in human and livestock production. In this research article, we decided to investigate the phytochemical analysis, antioxidant, anti-inflammatory, cytotoxicity, and anticoccidial activity of the herbal product APS. Initial phytochemical testing for the APS active principles was conducted and recorded [34].

Phytochemical screening of *Astragalus* polysaccharides material

The results of phytochemical screening agreed with the results of Gong *et al*. [9], who reported that APS included polysaccharides, saponins, amino acids, flavonoids, isoflavonoids, alkaloids, and astragalosides (Tables 1 and 2, Fig. 1).

Antioxidant activity by the 1, 1-diphenyl-2-picryl hydrazyl radical scavenging method

Results for the DPPH radical scavenging method were used to test the antioxidant activity of APS. At the various tested concentrations (100, 50, 25, 12.5, 6.25,

Table 1 Preliminary phytochemical tests on *Astragalus* polysaccharides

Experiments	Result
(a) Alkaloid	
Mayer's test	Yellow cream precipitate (+ve)
Wagner's test	Reddish brown precipitate (+ve)
Dragendorff 's test	Orange precipitate (+ve)
(b) Flavonoids	
Lead acetate test	Yellow precipitate (+ve)
(c) Carbohydrates/glycosides	
Fehling's test	Red precipitate (+ve)
Benedict's test	Red orange precipitate (+ve)
(d) Saponins	
Foam test	Persistent foam for ten minutes (+ve)
(e) Tannins	Ink color (+ve)
(f) Resins	No turbidity formed (-ve)

+ve, positive; –ve, negative.

3.12, 1.56, 0.78, 0.39, and 0.19 μg/ml), APS showed antioxidant activity that was comparable to that of normal ascorbic acid (vitamin C). For all concentrations examined, the percentage of antioxidant activity increased in a dose-dependent manner. Both extracts were found to have considerable DPPH radical scavenging abilities (Fig. 2).

Determination of LD_{50} of *Astragalus* polysaccharides extract

Acute toxicity studies on APS in mice were conducted using oral administration. Several groups of mice were used in pilot experiments to determine the LD_{50} and record toxicity symptoms. Deaths were not observed after oral administration of APS in mice at doses of 1, 3, 5, and 10 g/kg body weight over 72 h at the doses tested. The animals displayed no symptoms of toxicity at these doses. The median fatal dose (LD_{50}) for both samples was discovered to be higher than the highest dose examined. These findings indicate that the tested samples are extremely safe.

Table 2 Determination of total phenolic and total flavonoid content

	APS extract
Total phenols (μg gallic acid equivalent/g dry tested sample)	170.18
Total flavonoids (μg rutin equivalent/g dry tested sample)	220.41

APS, *Astragalus* polysaccharides.

Determination of the anti-inflammatory activity of *Astragalus* polysaccharides extract *in vitro*

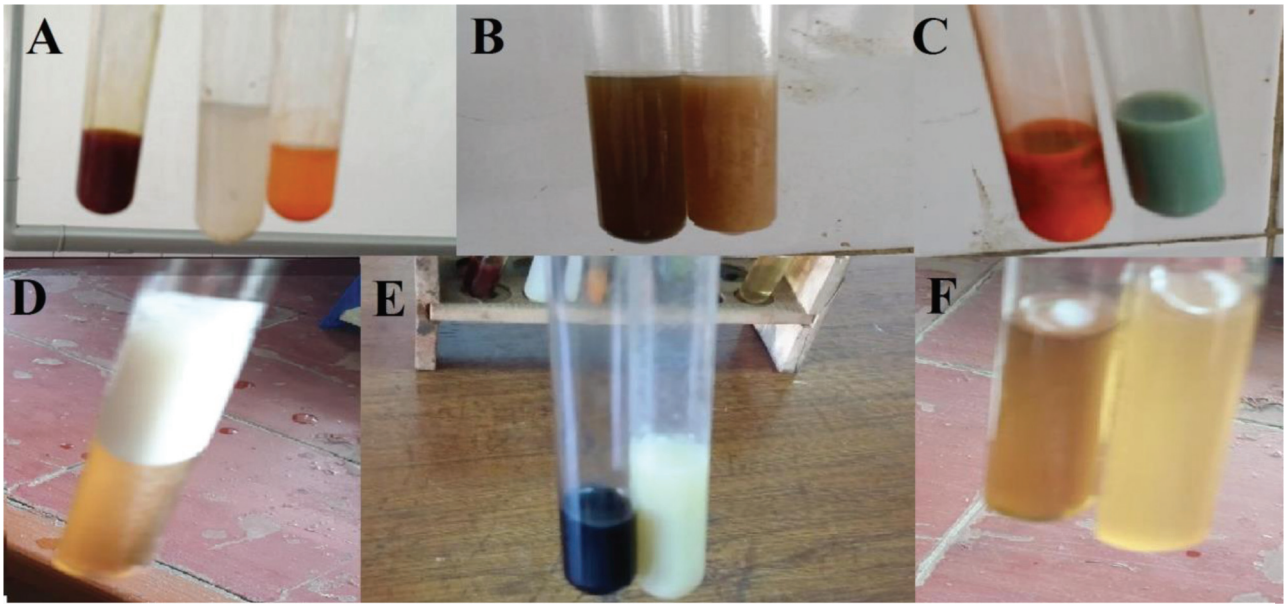
In vitro COX-1/COX-2 inhibition

The results are shown in Table 3 as the selectivity index (SI), or the chemical concentration (IC_{50}) that inhibits an enzyme by 50%. Results of *in vitro* anti-inflammatory activity demonstrated that the power of herbal medicine in the form of APS inhibited the COX-1 and COX-2 15.6 and 0.048 (IC_{50}, μm) compared with the standard drug celecoxib 16.5 and 0.061 (IC_{50}, μm) [10]. The lipid peroxidation of EA. hy926 cells was decreased by the antioxidant APS [35], which neutralizes oxygen-free radicals inside the cells. The results of the antioxidant and anti-inflammatory activities supported their conclusions. APS may be effective in reducing inflammation, NF-B p65 activation, and the production of cytokines including IL-8 and ICAM-1. APS may have biological activity such as antioxidant and anti-inflammatory effects, according toMeng *et al.* [12] and Yu *et al.* [36] (Figs 3 and 4).

Impact of the *Astragalus* polysaccharides on coccidia

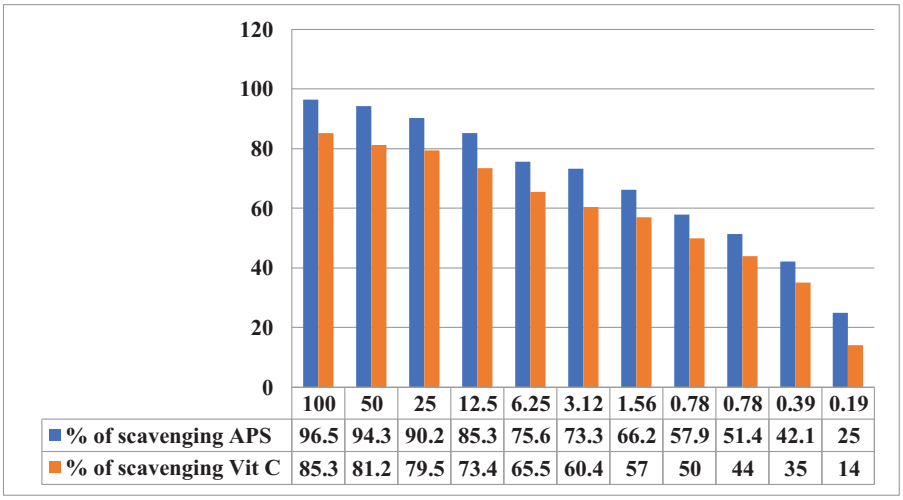
Chicken coccidiosis causes significant financial losses in addition to high rates of morbidity and death in poultry operations [5]. The cellular and humoral immune responses of chickens infected with *E. tenella* are profoundly impacted by APS [10]. When APS were used, it was discovered that they prevented oocyst sporulation, where they have powerful *in vivo* and *in vitro* anticoccidial effects [19]. According to Qiao *et al.* [37], the polysaccharides obtained from *A.*

Figure 1



Phytochemical screening on *Astragalus membranaceus* extract where all tests gave positive results for: (a) alkaloids, (b) flavonoids, (c) carbohydrates and glycosides, (d) saponins, (e) tannins, and negative for (f) resins.

Figure 2



Antioxidant activity of APS (the DPPH method). APS, *Astragalus* polysaccharides; DPPH, 1, 1-diphenyl-2-picryl hydrazyl.

Table 3 Anti-inflammatory activities (IC_{50}, μm) of *Astragalus* polysaccharides and celecoxib (*in vitro* COX-1 and COX-2)

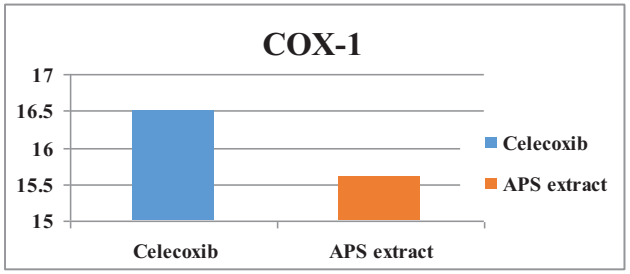
	COX-1 (IC_{50}, μm)	COX-2 (IC_{50}, μm)
Celecoxib	16.7±0.46	0.058±0.007
APS extract	15.58±0.3	0.042±0.008

APS, *Astragalus* polysaccharides.

membranaceus might raise broilers' body weight gain, decrease their feed conversion ratio, and improve their intestinal health. In addition, broilers' cecal microbiota became richer and more diverse after receiving APS and GPS food supplements, which also drastically changed the makeup of microbial communities. Considering this, the objective of the current investigation was to evaluate the anticoccidial potency of APS [38]. In this study, the generated APS's anticoccidial action was assessed. The current study evaluated the inhibitory effects of APS on unsporulated and sporulated oocysts of five distinct *Eimeria* spp. to estimate the APS LC_{50} and LC_{90}.

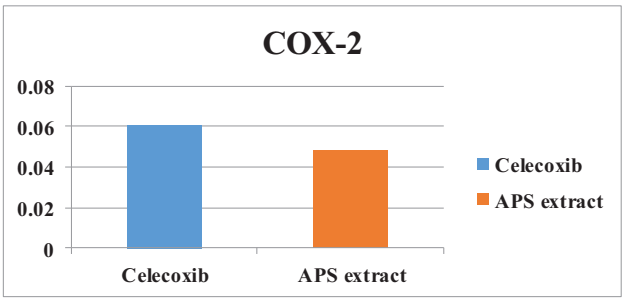
To investigate the APS's coccidiocidal effect, unsporulated immature *Eimeria* spp. oocysts undergo exposure to 10, 25, and 50 mg of the APS for 1, 3, 6, 9, 12, 24, and 36 h. The data in Table 4; Fig. 5 demonstrated a causal relationship between the rate of sporulation inhibition, APS concentrations, and exposure time. Only 22.3±1.5% of the oocysts subjected for 1 h died after being subjected to 10 mg of APS. This gradually increased to 85.3±1.9% after 36 h of exposure. Simultaneously, 25 mg revealed 35.1 ±0.9% mortalities, which increased to 96.8±1.3% after 36 h. While the highest mortality rate was recorded after 50 mg exposure, it was 46±0.9% after 1 h and increased to 95.4±0.7%, then 99.1±06% after 48 and

Figure 3



In vitro COX-1 anti-inflammatory activities (IC_{50}, μm) of APS extract and celecoxib. APS, *Astragalus* polysaccharides.

Figure 4



In vitro COX-2 anti-inflammatory activities (IC_{50}, μm) of APS extract and celecoxib. APS, *Astragalus* polysaccharides.

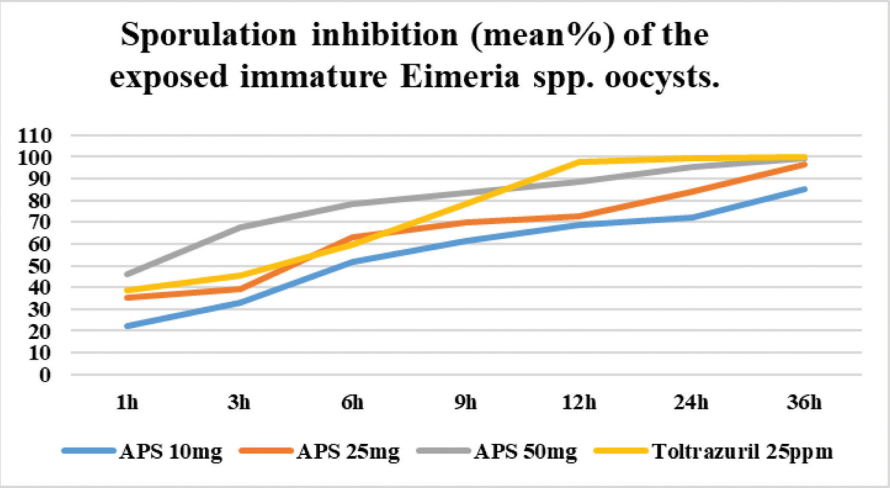
36 h, respectively. Toltrazuril 2.5% at a concentration of 25 ppm, however, revealed inhibition of 97.9±0.8% after 12 h of exposure time with inhibition of sporulation of the control neglected during calculation at each time. These findings supported those of Farag and Alagawany [33]. The ability of the employed APS to reduce the infectivity of the

Table 4 The mean percentage of inhibition of sporulation in subjected immature *Eimeria* spp. oocysts

	Eimeria spp. oocysts' mean±SD of sporulation inhibition upon exposure to						
Tested conc.	1 h	3 h	6 h	9 h	12 h	24 h	36 h
APS 10 mg	22.3±1.5^{b}	33±1.7^{b}	52±2.1^{b}	61.3±2.7^{b}	68.7±1.5^{bc}	72±1.7^{b}	85.3±1.9^{ab}
APS 25 mg	35.1±0.9^{ab}	39.3±1.3^{b}	63.2±1.7^{b}	69.7±1.4^{b}	72.6±1.2^{bc}	84.3±1.7^{ab}	96.8±1.3^{a}
APS 50 mg	46±0.9^{a}	67.8±0.9^{a}	78.6±1.1^{a}	83.5±0.8^{a}	88.7±0.9^{b}	95.4±0.7^{a}	99.1±0.6^{a}
Toltrazuril 2.5% 25 ppm	38.7±0.3^{ab}	45.3±0.3^{b}	59.5±0.7^{b}	78.3±0.8^{b}	97.9±0.8^{a}	99.2±0.5^{a}	-
Control –ve				0.00			

APS, *Astragalus* polysaccharides. *Concentration before these concentrations did not induce any mortalities. *Data reported as mean±SD, each column with different letters is statistically significant at *P* value less than or equal to 0.05 (one-way analysis of variance).

Figure 5



Calculating the LC_{50} and LC_{90} as well as demonstrating the effects of various APS doses and exposure times on the inhibition of sporulation in the subjected immature oocysts. APS, *Astragalus* polysaccharides.

Table 5 Calculated LC_{50} and LC_{90} of *Astragalus* polysaccharides versus *Eimeria* spp. unsporulated oocysts

LC_{50}	LC_{90}
10 mg/6 h	–
25 mg/4.5 h	25 mg/29 h
50 mg/1 h	50 mg/12 h

subjected oocysts has repeatedly been highlighted by several writers, including Shahrajabian *et al.* [39] and Abbas *et al.* [39], as a sure sign of the effectiveness of therapy and control of the coccidiosis disease.

Table 5 and Fig. 5 show the calculated LC_{50} and LC_{90} for each tested concentration. The LC_{50} values were 10 mg/6 h, 25 mg/4.5 h, and 50 mg/1 h. The LC_{90} at

Table 6 The level of DNA destruction in *Eimeria* spp. oocysts after 24 h of subjection to various *Astragalus* polysaccharides concentrations were examined, along with comet parameters

Different concentration	% sporulation inhibition	% DNA damage	Tail's length (μm)	% Tailed DNA	Tail's moment (μm)
Unsporulated oocysts					
10 mg/24 h	72±1.7^{b}	13.4±0.8^{bc}	8.9±0.7^{c}	7.9±0.8^{c}	0.57±0.05^{c}
25 mg/24 h	84.3±1.7^{ab}	16.8±0.6^{b}	9.7±0.9^{c}	9.9±0.8^{c}	0.89±0.04^{ab}
50 mg/24 h	95.4±0.7^{a}	19.2±0.4^{a}	10.8±1.1^{ab}	11.8±0.5^{ab}	0.95±0.03^{a}
Sporulated oocysts					
25 mg/29 h	LC_{90}	17.9±0.8^{a}	10.1±0.8^{ab}	10.2±0.7^{b}	1.07±0.05^{ab}
50 mg/12 h	LC_{90}	19.6±0.3^{a}	11.3±0.4^{a}	12.2±0.3^{a}	0.92±0.02^{a}
Toltrazuril 25 ppm/24 h	97.9±0.8^{a}	19.1±0.7^{a}	12.1±0.3^{a}	12.5±0.2^{a}	1.09±0.08^{a}
Negative control	0.0	3.6±0.2	3.3±0.4	2.8±0.8	0.25±0.03
P value			≤0.05		

Data represented by mean±SEM, a column with different letters are statistically significant at *P* less than or equal to 0.05 (one-way (analysis of variance).

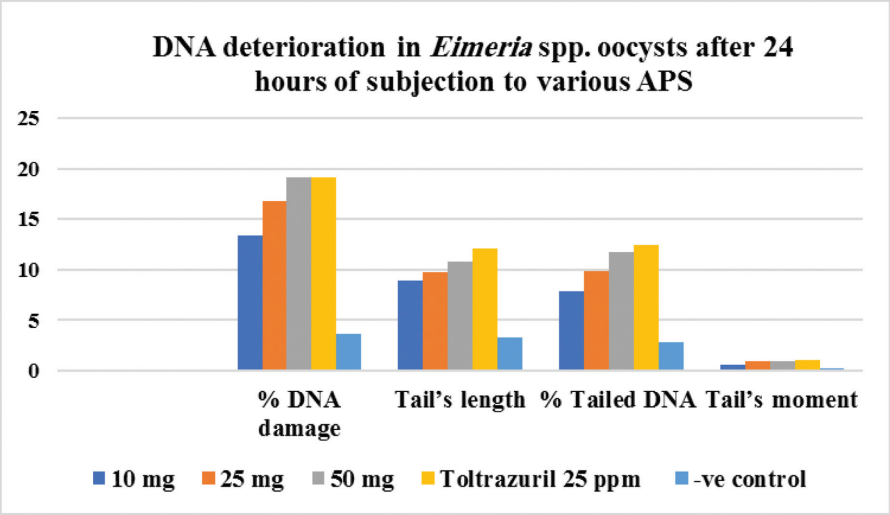
25 and 50 mg concentrations only inhibited the sporulation of the subjected immature oocysts after 29 and 12 h, respectively.

DNA deterioration of the subjected oocysts (comet assay)

The comet assay was used to examine the DNA damage in subjected and control *Eimeria* spp. oocysts. DNA damage was observed after 24 h of exposure in immature oocysts subjected to the three APS concentrations tested, in sporulated oocysts subjected to LC_{90} (25 mg/29 h and 50 mg/12 h), as well as in control (Toltrazuril 25 ppm/12 h) and negative control nonsubjected oocysts (Table 6 and Figs 6 and 7).

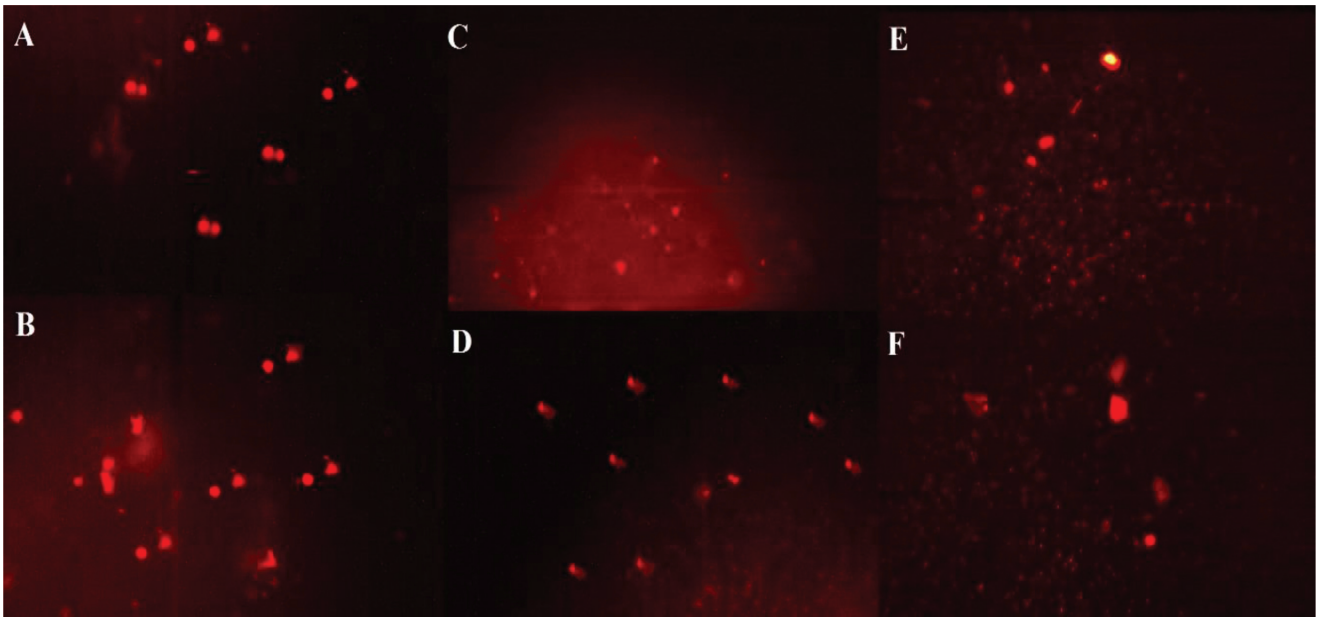
The variations in tail's length (μm), the amount of DNA in the tail segment (%DNA), and the tail's moment (μm) are also indicators of DNA genotoxic damage, and the results demonstrated a clear

Figure 6



The level of DNA deterioration in *Eimeria* spp. oocysts after 24 h of subjection to various APS concentrations. APS, *Astragalus* polysaccharides.

Figure 7



Comet assay demonstrates the damage in the DNA of *Eimeria* spp. oocysts subjected to APS; (a, b) control nonsubjected oocysts; (c, d) low level of damage recorded in that subjected to 10 and 25 mg/24 h; and (e, f) high damage of oocysts subjected to 50 mg and Toltrazuril 25 ppm/24 h. APS, *Astragalus* polysaccharides.

relationship between the increase in APS dose and the level of overall DNA damage. When the APS dose was increased from 10 to 50 mg, sporulation inhibition increased from 72.7 to 95.4% when the mobility of DNA fragments was examined using agarose gel electrophoresis. The amount of DNA damage increased significantly ($P \leq 0.05$) from 13.4±0.8 to 19.2±0.4%, and the mean tail's length (μm) increased from 8.9±0.7 to 10.8±1.1. Furthermore, the amount of DNA in the tail increased from 7.9 ±0.8 to 11.8±0.5% in oocysts subjected to 50 mg. The values progressively increased with the tested potassium dichromate concentrations ranging from 10 to 50 mg/ml (Table 6). The same results were obtained in sporulated oocysts subjected to LC_{90}.

The mean values of DNA destruction in unsporulated oocysts subjected to 50 mg and sporulated oocysts subjected to LC_{90} show no significant difference ($P \leq 0.05$) when compared with those obtained after exposure to the control reference drugs (Toltrazuril 25 ppm). Simultaneously, significant very low destruction (3.6±0.2% in DNA) and the other estimated parameters were recorded in nonsubjected control oocysts (Table 6 and Fig. 7).

The DNA damage in subjected and control oocysts was also evaluated using the comet assay. The results of the current investigation demonstrated that APS prevented immature *Eimeria* spp. oocysts from sporulating. This rose as the APS concentration and exposure time increased. According to Abdel-Tawab *et al.* [19] and Abdelaziz *et al.* [40], who found that the *Astragalus* root extract, at doses of 50 mg/kg, can boost the immune system and that it has considerable anticholinesterase and antioxidant action, the 50 mg/kg APS dose was found to be safe and free of any obvious toxicity or side effects. Exposure to *Eimeria* spp. oocysts increased the inhibitory rate of sporulation in a manner consistent with that previously observed by Taha *et al.* [4], Abdel-Tawab *et al.* [19], and Hartady *et al.* [41] described the ability of herbal products to induce DNA damage in exposing agents, stating that changes in tail length investigated by comet assay in comparison to the control nonsubjected stages reflected marked DNA damage, which increased with the increase in APS extract concentration.

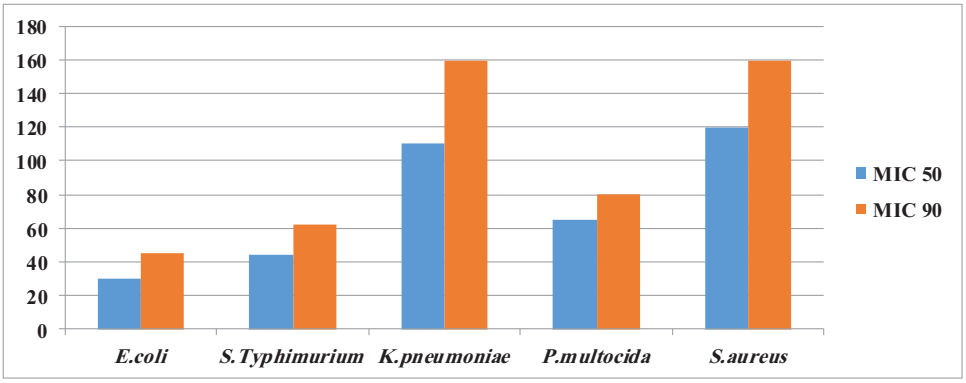
Determination of minimum inhibitory concentration and minimum bactericidal concentration of *Astragalus* polysaccharides against selected bacteria

Results of antimicrobial activity showed that MIC_{50} and MIC_{90} were (30, 45 μg/ml), (44, 62 μg/ml), (110, 160 μg/ml), (65, 80 μg/ml), and (120, 160 μg/ml) against *E. coli*, *S. typhimurium*, *K. pneumoniae*, *P. multocida*, *and S. aureus*, respectively. The obtained results of antimicrobial activity showed that MBC_{50} and MBC_{90} were (80, 130 μg/ml), (110, 140 μg/ml), (200, 410 μg/ml), (150, 280 μg/ml), and (250, 340 μg/ml) against *E. coli*, *S. typhimurium*, *K. pneumoniae*, *P. multocida*, *and S. aureus*, respectively. These results agreed with those of Lai *et al.* [42], who reported that APS resulted in mild inhibition of five pathogenic bacteria; also, in most conditions, *Astragalus* can be administered safely in addition to conventional antibiotics when there are normal gut bacteria present (Figs 8 and 9, Tables 7 and 8).

Conclusion

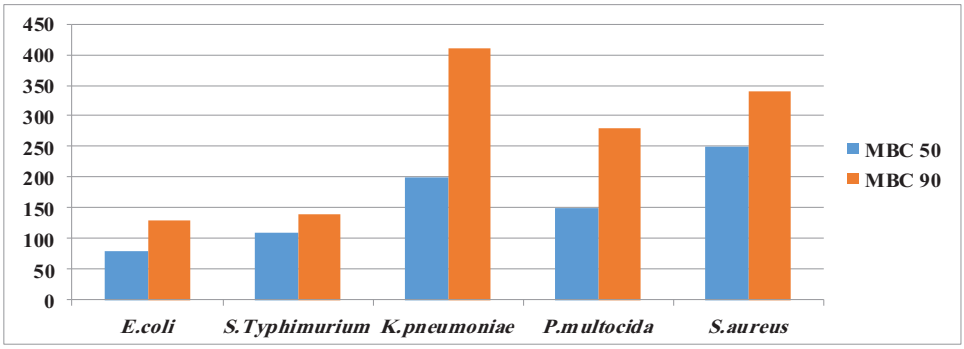
The tested herbal APS extracts proved to have an effective phytochemical compound (alkaloids, flavonoids, and glycosides), antioxidant, anticoccidial, and antibacterial activities. The tested APS demonstrated a potential new formulation of an

Figure 8



MIC_{50} and MIC_{90} (μg/ml) values of APS against selected bacteria. APS, *Astragalus* polysaccharides; MIC, minimum inhibitory concentration.

Figure 9



MBC_{50} and MBC_{90} (μg/ml) values of APS against selected bacteria. APS, *Astragalus* polysaccharides; MBC, minimum bactericidal concentration.

Table 7 Determination of minimum inhibitory concentration$_{50}$ and minimum inhibitory concentration$_{90}$ (μg/ml) values

	MIC (μg/ml)	
	MIC_{50}	MIC_{90}
Escherichia coli (ATTC 25922)	30	45
Salmonella typhimurium (ATTC 13311)	44	62
Klebsiella pneumoniae (ATTC 10031)	110	160
Pasteurella multocida (ATTC 43137)	65	80
Staphylococcus aureus (ATTC 25923)	120	160

MIC, minimum inhibitory concentration.

Table 8 Determination of minimum bactericidal concentration$_{50}$ and minimum bactericidal concentration$_{90}$ (μg/ml) values

	MBC (μg/ml)	
	MBC_{50}	MBC_{90}
Escherichia coli (ATTC 25922)	80	130
Salmonella typhimurium (ATTC 13311)	110	140
Klebsiella pneumoniae (ATTC 10031)	200	410
Pasteurella multocida (ATTC 43137)	150	280
Staphylococcus aureus (ATTC 25923)	250	340

MBC, minimum bactericidal concentration.

effective, safe, economical, and environmentally friendly coccidiocidal material capable of inducing significant genotoxic damage on the DNA level of the zygote and its sporocysts. It significantly reduced immature and mature *Eimeria* spp. oocyst sporulation and infection. The efficacy of this product as a coccidiocidal and bacteriocidal medication in a large group of experimentally infected chicken is currently being evaluated based on the promising *in vitro* data obtained in the present study.

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Authors' contributions: All of the authors agreed on the study's goal, and Marwa M. Khalifa and Reem M. Ramadan carried out the current protocol's steps and interpreted the results. Ahmed Orabi and Ehab A. Fouad test APS's antimicrobial efficacy, where Fady S. Youssef created and carried out the various APS tests. This manuscript was written, revised, and approved by the authors.

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Conflicts of interest

There are no conflicts of interest.

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