Hematological profile, rumen fermentation, antioxidant state, and immune response of Egyptian Nubian goats fed on *Astragalus membranaceus* root extract supplemented ration

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Background
In recent years, *Astragalus membranaceus* extract has been widely used in animals due to their antimicrobial activities, ability to enhance immunity, and antioxidant functions.
Objective
We aimed to determine the antioxidant and inhibitory activities of *A. membranaceus* root powder (AMP) and its cytotoxicity and effects on hematological profile, rumen fermentation, antioxidant status, and immune response in Egyptian Nubian goats.
Materials and methods
Twenty-five goats are used in this study were received 20 g/animal/day of AMP mixed with their basic diet for 28 days. The study involved measuring the antioxidant activity of AMP using the 2-diphenyl-1-picrylhydrazyl radical scavenging assay and determining the viability and cytotoxicity percentage using the methyl-thiazolyl tetrazolium protocol.
Results
After 14 and 28 days of the daily feeding with 20 g of AMP, there was a significant increase in hematological profile, leukocyte count, total volatile fatty acid, and rumen ammonium concentrations with an enhancement in protozoal activity. Also, there was an increase in catalase and total antioxidant capacities along with promoting immunoglobulin (A, M, and G) contents with no significant effect on the insulin level compared with 0 days. Malondialdehyde contents decreased significantly. For all examined concentrations, *A. membranaceus* showed antioxidant and anti-inflammatory activities. It also showed a high cytotoxicity percentage in cancer cells.
Discussion and conclusions
A. membranaceus root extract supplementation significantly increases hematology parameters and rumen fermentation, and improves immune status and antioxidant activity both in-vitro and in live animals. It also exhibits potent cytotoxicity on cancer cells.

Keywords:
Astragalus membranaceus, Egyptian goats, hematology, immune and antioxidant status, rumen fermentation

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Introduction

For many years, it has been a common practice to use antibiotics to reduce disease susceptibility and to promote the growth and production of livestock. Many countries have forbidden the use of these antibiotics because they pose a high risk to food safety. Due to the growing demand for healthy animal products in the consumer market, natural products have been developed as an alternative to drugs to enhance growth and immune performance. Antimicrobial, immune-ehancing, and antioxidant effects of natural herbal plants have made them popular as feed supplements in recent years [1,2].

A traditional Chinese herbal medicine known as Huangqi has been used for centuries as *Astragalus membranaceus* dried roots. Among the numerous bioactive molecules of this herb are 25 amino acids, 11 monosaccharides, 13 triterpenes and flavonoids, nine isoflavonoids, 24 polysaccharides, 13 monosaccharides, 13 triterpenes and flavonoids, nine isoflavonoids, and 25 amino acids [3,4].

A. membranaceus root has also been shown to have anti-inflammatory, antimicrobial, antiviral, and antioxidant properties, as well as growth and immunity-enhancing properties [5,6]. Pharmacological studies have revealed

that the most essential constituent of Astragalus is its polysaccharide *Astragalus* polysaccharide (APS), which has numerous pharmacological properties, including enhancing immunity and stimulating the production of cytokines and immunoglobulins. It also encourages apoptosis in cancer cells and inhibits the proliferation and transfer of tumor cells [7].

There is an increasing demand for preventing oxidative stress in fattening animals, as well as promoting animal health and production. Ruminant meat producers will benefit greatly from A. *membranaceus*, which stimulates immune function and antioxidant activity.

Using *A. membranaceus* root extract as a feed additive in Egyptian Nubian goats is aimed at determining its effect on hematology, rumen fermentation, antioxidant status, and immune responses

Materials and methods

An extract of *Astragalus membranaceus* root powder was prepared and tested for its phytochemical properties

Extraction was performed according to a method described by Zhu *et al*. [8]. *A. membranaceus* extract was analyzed using traditional phytochemical methods according to the method described by Pant *et al*. [9].

Analyzing *Astragalus membranaceus* roots for phenolic content and flavonoids

According to the methods described by Baba and Malik [10], total phenolic content was measured. To determine total flavonoids, aluminum chloride colorimetry was used as described by Baba and Malik [10].

Astragalus membranaceus anti-oxidative activity *in vitro*

The antioxidant properties of *A. membranaceus* were compared with those of regular vitamin C using the method mentioned by Khedr *et al*. [11]

Cyclooxygenase enzyme 1 and cyclooxygenase enzyme 2 inhibition by *Astragalus membranaceus in vitro*

According to Kulmacz and Lands [12], indomethacin (the reference standard) and *A. membranaceus* extract were also tested at different concentrations for their IC50 values on cyclooxygenase enzyme 1,2 (COX-1 and COX-2).

Astragalus membranaceus cytotoxicity tests on cells (methyl-thiazolyl tetrazolium protocol)

The method described by Riss and Moravec [13] was used, and the morphological assay is associated with large-scale changes in cell viability and morphology, including changes that take place in the cytoskeleton or at the cell surface as described by Mekky *et al*. [14].

Experimental design, diet, and animals

IACUC at Cairo University approved the questionnaire and methodology for our experiment (Ethics approval number: Vet CU 03162023747). A random sample of 25 adult female Nubian goats, 3–4 years old, 40–50 kg (by 0.5 kg), not pregnant or lactating, was used in this study. In the Giza Government, Egypt, goats were housed on a private farm. Pellets and hay were provided twice a day as a basic diet, and water and trace minerals were available at all times. Fourteen days after adapting, the goats joined the experiment. Over 28 days, they were fed *A. membranaceus* root powder (AMP) in a 20-g daily dose mixed with their basic diet.

The sampling processes

On days 0, 14, and 28, blood samples were drawn from the jugular vein into fresh vacuum tubes without anticoagulants, as well as ETDA-containing tubes. A 450 nm goat insulin enzyme-linked immunosorbent assay (ELISA) kit (LSBio, Seattle, Washington, USA) was used for analyzing insulin in plain tubes centrifuged at 3000 rpm for 15 min according to manufacturer's instructions. With ELISA kits from MyBioSource (San Diego, USA), we measured superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), total antioxidant capacity (T-AOC), and serum immunoglobulin (IgA, IgG, and IgM). With ELISA kits from Bio-Diagnostic (Giza, Egypt), we measured catalase (CAT) and malondialdehyde (MDA).

The automated hematological analyzer (Mindray BC-2800 VET, Guangdong, China) was used to determine total erythrocyte count, hemoglobin, platelet count, and total leukocyte count using EDTA-containing test tubes.

A stomach tube (Anscitech, Wuhan, China) was used to collect rumen juice from each goat on days 0, 14, and 28. To decrease saliva contamination, the first 30 ml of each goat's sample was discarded. A pH meter (M90; Corning Inc., Corning, New York, USA) was used to measure the pH of rumen juice, and then the juice was strained through four layers of cheesecloth and stored at −80°C for further analysis. Volatile fatty acids were determined using a chromatograph (BEIFEN SP-3420A, Beijing, China) following the method of Zhang *et al*. [15], and ammonia-N (NH_3-N) was determined following the method described by Hristov *et al*. [16].

Analysis of statistical data

Based on the illustrated data, the mean and SD are expressed as mean±SD. Excel 2013 was used to draw graphs. With SPSS 27 (IBM, New York, New York, USA), the difference was considered significant when *P* value is 0.05 using analysis of variance.

Results

Phytochemical screening

Pharmacological screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins in *A. membranaceus* extract but not resin.

According to Fig. 1, *A. membranaceus* extract has ideal phenol and flavonoid contents (193.15 g gallic acid equivalents/g dry sample and 261.6 g rutin equivalents/g dry sample, respectively).

Antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method

A. membranaceus was tested for antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method. Concentrations tested ranged from 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19 g/ml. Compared with ascorbic acid, *A. membranaceus* showed comparable antioxidant activity. There was a dose-dependent increase in antioxidant activity for all concentrations examined. According to Fig. 2, both extracts are highly effective at scavenging 2, 2-diphenyl-1-picrylhydrazyl radicals.

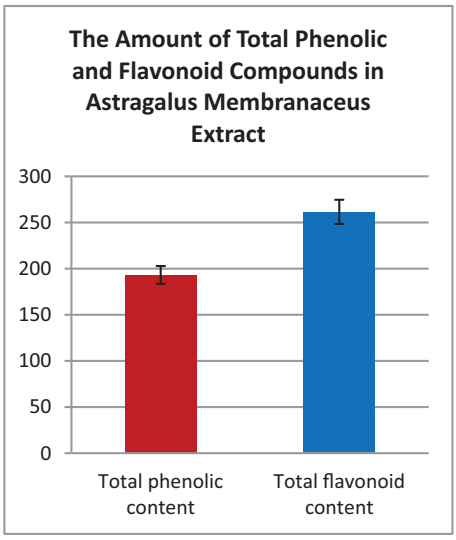
In vitro inhibitory effect of *Astragalus membranaceus* on cyclooxygenase enzyme 1,2

As shown in Figs 3–6) *A. membranaceus* extract exhibited strong inhibitory and anti-inflammatory activities on both COX-1 and COX-2 as compared with the tested drug (indomethacin).

Methyl-thiazolyl tetrazolium protocol was used to determine cytotoxicity of *Astragalus membranaceus* in HepG2 cells at different concentrations: 1000, 500, 250, 125.5, 62.5, and 31.25 µg/ml

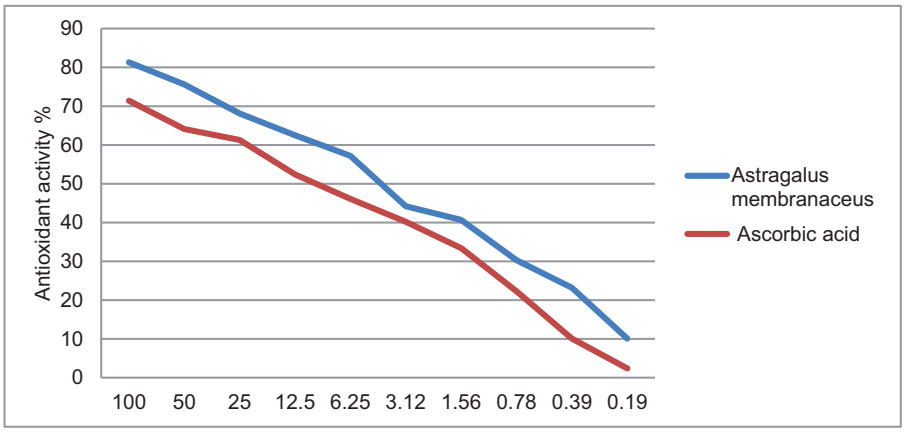
A. membranaceus was tested for its ability to terminate tumor cell growth using the cytotoxicity test. At a well-known concentration, a compound was considered active if it could terminate 50% of tumor cell proliferation. For cytotoxicity measurement, methyl-thiazolyl tetrazolium (MTT) was used, a method that can be reproducible with low variability in dose–response curves. Response criteria should be proportional to cell numbers, and the resulting information should correspond to the appearance of the cells. It is considered anticancer activity when a compound inhibits 50% of the proliferation of cancer cells with a concentration of less than 200 mg/ml (IC50: 200 mg/ml).

Figure 1



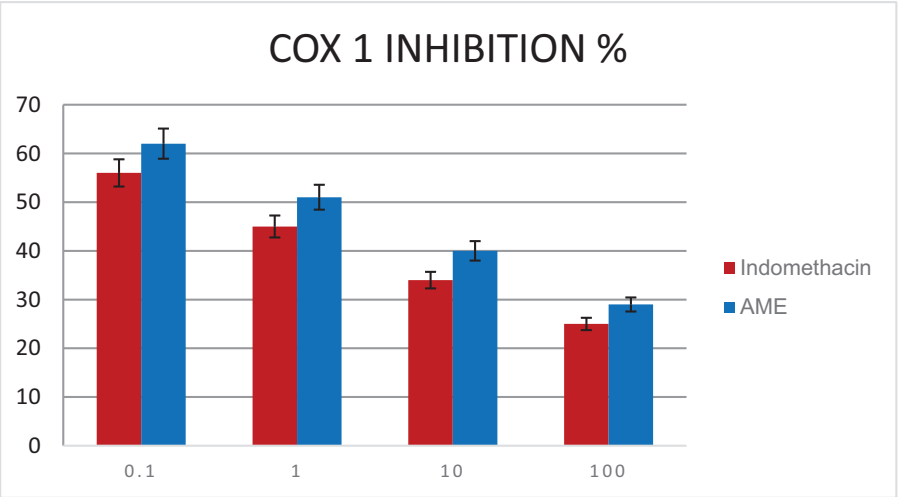
The amount of total phenolic and flavonoid compounds in the extract of *Astragalus membranaceus*.

Figure 2



Represent DPPH radical scavenging method to test the antioxidant activity of *Astragalus membranaceus* compared with that of normal ascorbic acid at different tested concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19 g/ml). DPPH, 2, 2-diphenyl-1-picrylhydrazyl.

Figure 3



COX-1 (IC5O) and COX 2 (IC50) of AME compared with the standard drug (indomethacin). AME, *A. membranaceus* extract; COX-1, cyclooxygenase enzyme 1.

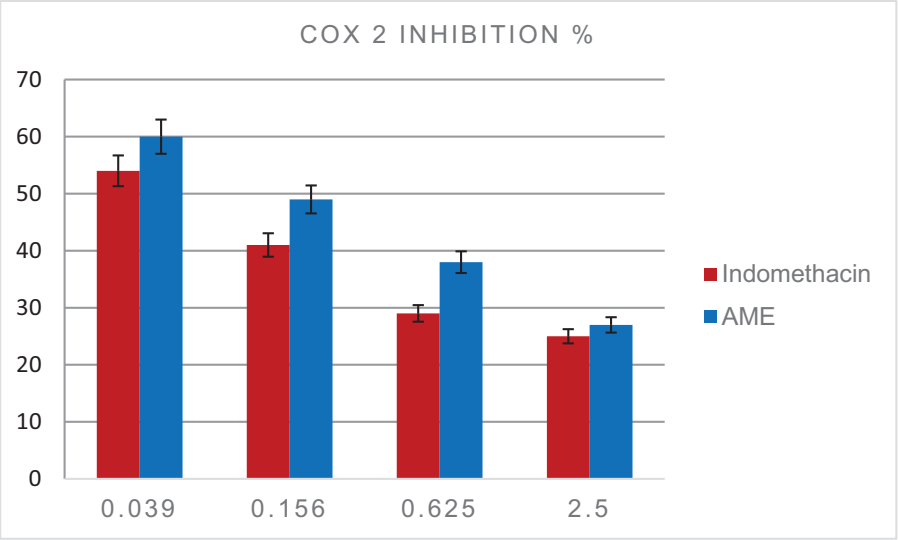
Based on the MTT protocol, the *A. membranaceus* extract showed significant cytotoxic activity on the HepG2 cell line. HepG2 which was treated with *A. membranaceus* extract for 24 h in 96-well plates showed a minimum toxic concentration at 62.5 µg/ml and a minimum concentration for viability percentage at 31.25 µg/ml concentration with IC50 51.3 as shown in Fig. 7.

When HepG2 cancer cells were treated with *A. membranaceus* samples for 24 h at different concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml, MTT microscopic imaging indicated large volume losses due to protein loss. Intracellular changes in ion concentration can be used to detect damage resulting from increased sodium and potassium permeability. In necrotic cells, chromatin flocculates, nuclear basophilia disappears, and nuclear enlargement occurs. In Fig. 8, apoptosis is represented by nuclear condensation, nuclear fragmentation, and cell shrinkage compared with controls (HepG2 cancer cells).

Hematological profile with feeding of *Astragalus membranaceus* supplemented ration

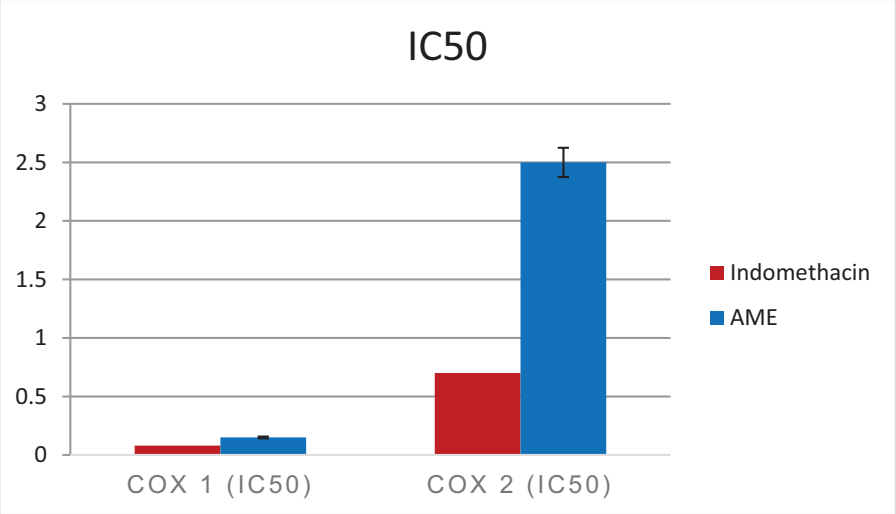
A. membranaceus powder was added daily to the basic diet for 28 days as a feed additive; the results showed significant improvement in hemoglobin content, red

Figure 4



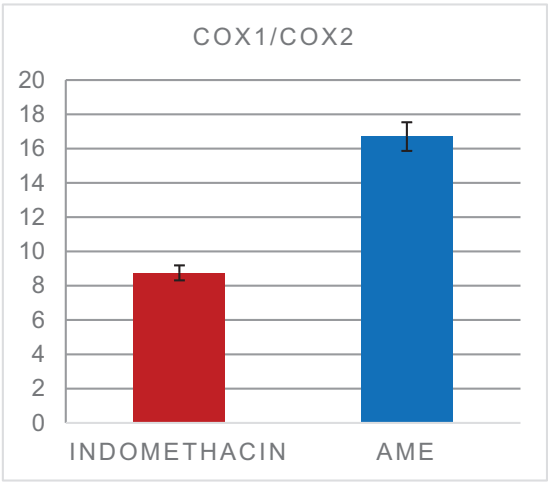
Inhibitory% of both indomethacin and AME on COX-2 at different tested concentrations (0.039, 0.156, 0.625, and 2.5 µg/ml). AME, *A. membranaceus* extract; COX-2, cyclooxygenase enzyme 2.

Figure 5



COX-1 (IC5O) and COX-2 (IC50) of AME compared with the standard drug (indomethacin). AME, *A. membranaceus* extract; COX-1, 2, cyclooxygenase enzyme 1, 2.

Figure 6



Selectivity index of both indomethacin and AME. AME, *A. membranaceus* extract.

blood cell count, packed cell volume%, platelet count, red blood cell index, and leukocyte count after 14 and 28 days of the daily feed of *A. membranaceus* powder of 20 g each, as detailed in Table 1.

In vivo antioxidant activity with feeding *Astragalus membranaceus* supplemented ration

Supplementing AMP promoted SOD activity as shown in Table 2. GSH-PX activity in serum was significantly affected by AMP supplementation. In addition, feeding of AMP increased CAT activity significantly. After 14 and 28 days of supplementation with AMP, T-AOC increased while MDA levels significantly decreased.

Effects of *Astragalus membranaceus* supplemented ration on insulin levels and immunity status

In Table 2, serum insulin and immune function are presented. IgA, IgM, and IgG contents in the serum were enhanced by daily AMP feeding to 20 g/animal over time without affecting insulin levels significantly.

Rumen parameters with feeding *Astragalus membranaceus* supplemented ration

Daily *A. membranaceus* supplementation increased rumen pH on days 14 and 28 compared with 0 days. Total volatile fatty acid (TVFA) concentrations in the rumen were higher after *A. membranaceus* feeding on days 14, and 28 compared with 0 days. On days 14 and 28, in addition to an increase in protozoal activity, there was a significant increase in rumen NH_3-N concentration (Table 2).

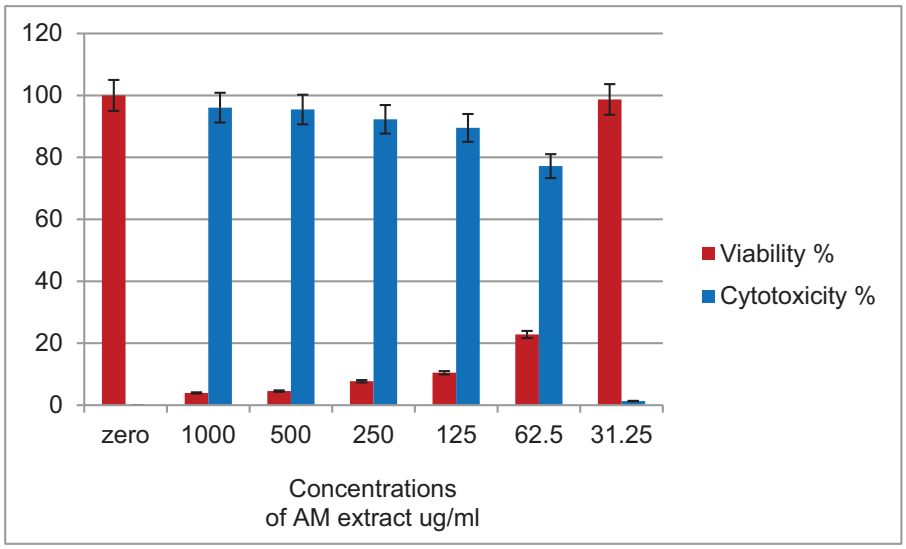
Discussion

In China, *A. membranaceus* grows wildly as a herbal medicine. They contain polysaccharides, amino acids, isoflavonoids, astragaloside, and lactic acid [17].

According to the results of phytochemical screening, *A. membranaceus* extract contains alkaloids, flavonoids, glycosides, tannins, and saponins.

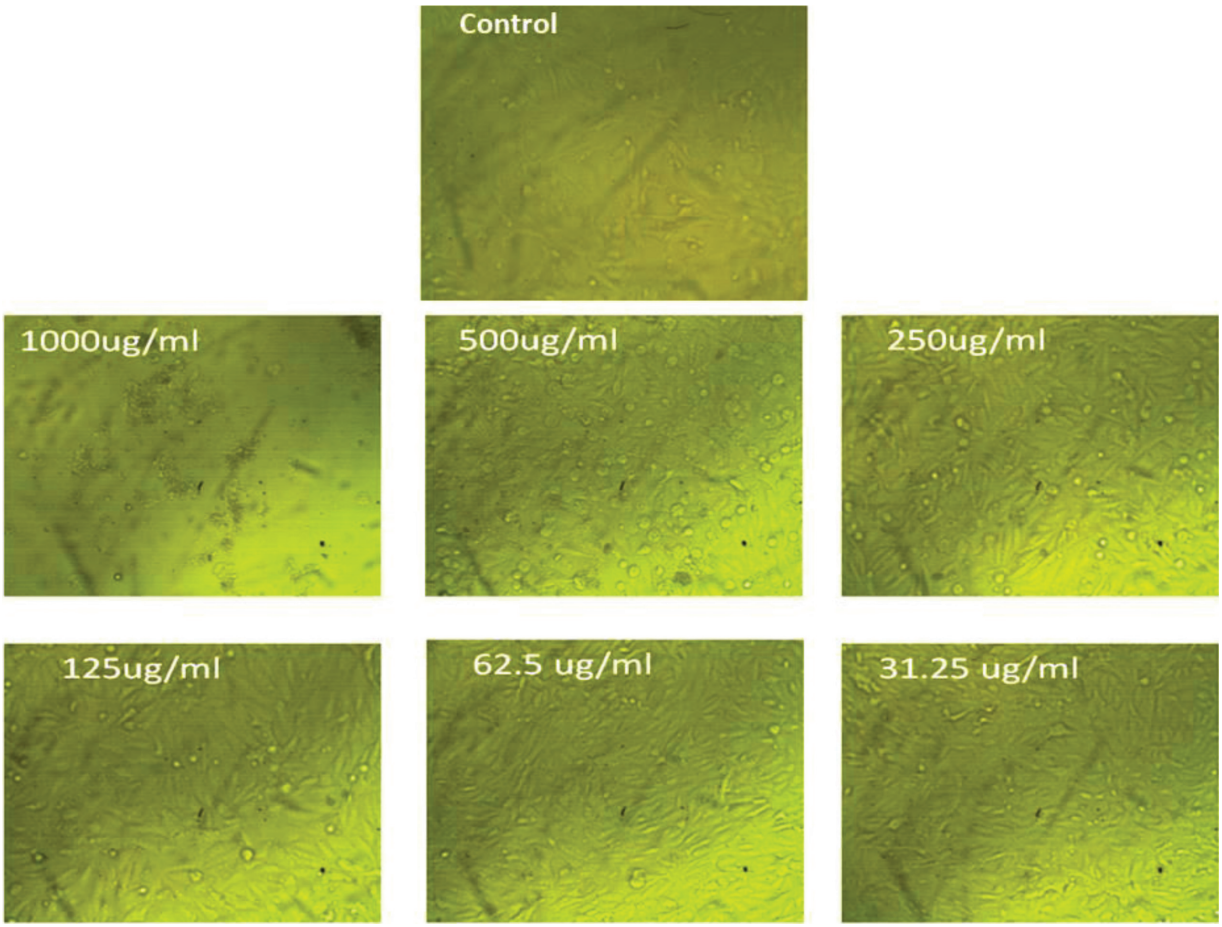
It has been reported by Gong *et al.* [18] that *A. membranaceus* contains polysaccharides, saponins, amino acids, flavonoids, isoflavonoids, alkaloids, and astragalosides. According to Zhang *et al.* [19], polysaccharides, saponins, and flavonoids, along with sucrose, phenolic acids, and amino acids, are the most biologically active components of *A. membranaceus*.

Figure 7



Viability %,cytotoxicity % and IC_{50}±SD of *Astragalus membranaceus* sample on HepG2 cancer cells at different concentrations 1000, 500, 250, 125.5, 62.5 and 31.25 µg/ml using methyl-thiazolyl tetrazolium protocol.

Figure 8



Cell viability and cytotoxicity % at the cell surface with *Astragalus membranaceus* different concentrations 1000, 500, 250, 125, 62.5, and 31.25 µg/ml compared to control (HepG2 cancer cells).

Table 1 Effect of *Astragalus membranaceus* root supplementation at 0, 14, and 28 days on hematological profile of normal Egyptian Nubian goats represented by mean±SD

Parameters	Egyptian Nubian goats (*N*=25) 0 day	Egyptian Nubian goats (*N*=25) 14 days	Egyptian Nubian goats (*N*=25) 28 days
Hemoglobin (g %)	8.6±0.12[a]	9.52±0.15[b]	9.56±0.29[b]
RBCs (10^6/mm^3)	10.7±0.13[a]	12.54±0.38[b]	13.1±0.6[b]
PCV %	24.82±0.35[a]	27.8±0.65[b]	29.14±0.84[b]
Platelets (10^3/mm^3)	482±27.55[a]	510.6±24.36[a]	543±14[a]
MCV (fl)	0.69±0.38 [a]	1.5±0.67 a	1.08±0.33[a]
MCH (pg)	23.3±0.42 [a]	21.4±0.68 [b]	23.27±0.44[b]
MCHC (g %)	8.08±0.11 [a]	7.4±0.27 [b]	7.59±0.22[b]
WBCs (10^3/mm^3)	12.35±2.8 [a]	12.9±2.4 [a]	13.12±1.6[a]

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell. [ab]Means within a row with different superscripts differ ($P<0.05$).

Table 2 Effect of *Astragalus membranaceus* root supplementation at 0, 14, and 28 days on insulin, antioxidant activity, serum immunity, and rumen fermentation of normal Egyptian Nubian goats represented by mean±SD

Parameters	Egyptian Nubian goats (*N*=25) 0 day	Egyptian Nubian goats (*N*=25) 14 days	Egyptian Nubian goats (*N*=25) 28 days
Hormone			
Insulin (Ulu/ml)	2.4±0.12	2.3±0.08	2.5±0.05
Antioxidant activity			
Superoxide dismutase (U/ml)	483.2±4.67[a]	499.28±5.6[b]	510.79±1.79[b]
Glutathione peroxidase (Mmol/l)	208.6±1.8[a]	309.3±3.8[b]	320.3±3.2[b]
Catalase (U/l)	147.63±6.46[a]	224.16±14.5[b]	266.96±4.8[c]
Malondialdehyde (nmol/ml)	5.79±0.12[b]	4.23±0.06[b]	3.53±0.04[a]
Total antioxidant capacity (Mm/l)	1.22±0.06[a]	1.5±0.09[b]	1.91±0.01[b]
Immunoglobulins			
Immunoglobulin A (mg/dl)	36.3±0.33[a]	54.3±3.9[b]	64.3±3.3[b]
Immunoglobulin M (mg/dl)	14.0±20.3[a]	24.6±15.2[b]	25±15.7[b]
Immunoglobulin G (mg/dl)	311.66±2.08[a]	355±0.57[a]	370±1.4[a]
Rumen parameters			
pH	6.1±0.03[a]	6.5±0.05[b]	6.6±0.04[b]
Ammonia (mmol/l)	12.1±0.09[a]	16.8±0.09[b]	18.02±0.19[c]
Total volatile fatty acid (mmol/l)	52.8±0.6[a]	61.5±0.9[b]	71.4±0.4[c]
Protozoal activity	++	+++	+++

[a–c]Means within a row with different superscripts differ ($P<0.05$).

Astragalosides, especially polysaccharides, are important bioactive components of *A. membranaceus*'s dried roots. *A. membranaceus* contains polysaccharides such as *Astragalus* I, II, and III. Glucose and arabinose are the major carbohydrates in *Astragalus* type I, while glucose, arabinose, and rhamnose are the major carbohydrates in *Astragalus* type II. D-glucose is the only component of *Astragalus* type III. In *A. membranaceus*, astragalin is the predominant polysaccharide. The cylcoastragenol structure of *A. membranaceus* enables it to be classified into seven groups of steroidal saponins called astragalosides. As stated in Ibrahim *et al.* [20], *A. membranaceus* contains flavonoids in glycosidic or free forms (flavones, flavolins, flavonols, and isoflavones).

There are many natural remedies available today for treating illnesses. Contrary to this, synthetic drugs are dangerous and have serious side effects that endanger public health. Drug resistance could develop as a result of consistent use of synthetic drugs. Herbal remedies are becoming increasingly popular because they have few side effects [21]. There are still many therapeutic plants and potential herbs that are being improved for use in human, animal, and poultry health, especially those with bioactive properties like antimicrobial, antioxidant, antidiabetic, antiparasitic, and anticancer [22]. According to new studies, *Astragalus* roots contain a variety of chemical constituents with various pharmacological and medical properties, such as antioxidant, antibacterial, and immune-boosting properties [23–25].

It is one of the most important pro-inflammatory cytokines produced during inflammation. As well as motivating immune cell activation and synthesis, it promotes apoptosis. Inhibition of the mucosal

barrier and increased permeability are the results of overexpression of these cells [26,27]. Cytokines and tumor necrosis factor-alpha activate and promote other inflammatory mediators, including COX-2, which impacts the integrity of the cell and tissue damage progression [28]. According to our results, *A. membranaceus* extract can inhibit COX-1 and COX-2 as well as anti-inflammatory effects. This is consistent with Adesso *et al*. [29] which found that *A. membranaceus* extract significantly decreased COX-2 expression in lipopolysaccharides+interferon-stimulated intestinal epithelial cells-6. *A. membranaceus* extract inhibited COX-2 expression by modulating the arachidonic acid cascade during inflammation, thus contributing significantly to its inhibitory effects on intestinal epithelial cells. In addition to inhibiting COX-2 expression and inflammation, *A. membranaceus* also exhibits anti-inflammatory effects [30,31]. In addition, our results are consistent with those of Ramadan *et al*. [32], who found that *A. membranaceus* extract decreased COX-1 and COX-2 activity.

Impact of *Astragalus membranaceus* on tumor cell viability and cytotoxicity

In the development of an antitumor drug, cytotoxic activity is evaluated during preclinical testing. A cytotoxicity evaluation is used to determine if the material contains any biological poisons [33]. Various plants have been used in the prevention and interference of carcinogenesis in the past few decades. Several intracellular signaling pathways, metastasis, systemic effects, and angiogenesis are blocked by plant polyphenols, which are the most potent antitumor materials [34].

In our study, *A. membranaceus* extract significantly inhibited HepG2 cancer cells at different concentrations with a high cytotoxicity at 1000 µg/ml of *A. membranaceus* extract concentration and a very low viability percent (3.94%). Cancer cells are apoptotic when exposed to APS as reported in many studies [35,36]. APS inhibits the cell cycle in the G1 stage of breast cancer cells (MCF-7 and 4T1) and promotes apoptosis. It is suggested in Wang [37] that APS inhibits ERK1/2 signaling by enhancing HepG2 cell differentiation into the G0-G1 and G0-M stages [38], resulting in the expression of BCL2 in HepG2 cells and the activation of caspase 3 [39], thereby promoting apoptosis among HepG2 cells. As in a study of Wu *et al*. [40], A549 cells under the effect of APS also showed an acceleration of the apoptosis process.

Treatment with APS increased pro-apoptosis BAX and caspase 8 levels, while anti-apoptosis BCL2 levels were reduced in human lung cancer H460 cells, increasing apoptosis [41]. Also, APS inhibits apoptosis in SGC7901 cells derived from human gastric cancers [42], probably by reducing the expression of BAX and BCL2 downstream of p53. Human early childhood leukemia HL-60 cells are also stimulated to undergo apoptosis by APS [43].

Effect of *Astragalus membranaceus* supplementation on hematological profile

The health status of the animals must first be evaluated before evaluating a new feed additive. An animal's hemo-biochemical composition is a good indicator of its health status. All blood count parameters were significantly improved compared with 0 days by adding the *A. membranaceus* powder to the basic diet for 14 and 28 days, suggesting that the animals' general health status improved along with the dietary treatment. Following daily feeding of 20 g of *A. membranaceus* powder for 14 and 28 days, most blood count parameters, such as hemoglobin, red blood cells, platelets, and leukocytes, were significantly increased.

There are very few published papers on *A. membranaceus*' effect on goat hemograms, but Lv *et al*. [44] found a similar effect in that *Astragalus* could reduce bone marrow cell apoptosis and enhance hemopoietic progenitor cells' differentiation into megakaryocytes and erythroids. A significant increase in colony-forming units-megakaryocyte was found after chemotherapy was administered to anemic mice treated with *Astragalus*, indicating that this herb could facilitate megakaryocyte hemopoiesis when bone marrow is downregulated [45]. Furthermore Chen *et al*. [46], reported that colony-forming units of erythroid cells, burst-forming units of erythroid cells, megakaryocytes, granulocytes, and macrophages proliferated following *Astragalus* therapy.

One of the most important components of the body's defense system is the WBC system, which is usually recruited from the blood to the infection site [47]. Evidence suggests that *Astragalus* may stimulate the production of white blood cells in the body as part of the immune-stimulating system [48]. In addition to its immune-enhancing effect and adaptogenic effect it promotes the recognition of viruses, bacteria, and even cancer cells by the immune system [49], It also stimulates the production of B lymphocytes, T lymphocytes, interleukin, and antibodies.

Antioxidant activity with *Astragalus membranaceus* supplementation

A major cause of animal diseases is oxidative stress [50]. A diet containing 50 g/kg of APS improves antioxidant activity by scavenging free radicals [51]. In addition, flavonoids and saponins derived from the extract have antioxidant properties [52]. Animals' oxidative status is largely determined by T-AOC and T-SOD [53].

With *A. membranaceus* powder supplementation, goats' T-SOD, CAT, and T-AOC activities were enhanced, but MDA concentrations were reduced, which indicated that *A. membranaceus* powder enhanced goats' antioxidant status. According to Youssef *et al.* [2], dietary *A. membranaceus* powder supplementation at levels of 50 g/kg also increased T-AOC, T-SOD, and CAT activities in the serum of weaned lambs. Endogenous antioxidant defense enzymes include GSH-PX, T-SOD, and CAT. Consequently, the increased activity of these enzymes following AMP supplementation resulted in goats being able to scavenge free radicals and reactive oxygen species with a lower level of MDA concentration, as indicated by a lower level of lipid peroxidation following AMP supplementation. AMP supplementation caused the serum antioxidant capacity to increase linearly due to its pooled antioxidant constituents. According to Ma *et al.* [54] and Kim *et al.* [55], *A. membranaceus* contains many natural biological components (such as polysaccharides, saponins, flavonoids, etc.) that have antioxidant properties. In addition to enhancing antioxidant activity, these exogenous antioxidants can also scavenge free radicals and upregulate endogenous antioxidant radicals [56,57]. In our study, AMP supplementation produced antioxidant effects in goats, suggesting that AMP may help alleviate goats' oxidative stress during periods of high production stress.

Insulin levels and immunity status as affected by *Astragalus membranaceus* supplementation

For the immune system to function properly, oxidative stress must be balanced with antioxidant levels [58]. As a result of their ability to decrease lipid peroxidation and scavenge free radicals, exogenous antioxidants are capable of reducing oxidative stress. According to previous studies, AMP can reduce immune stress and motivate the immune system [59]. In addition to increasing the production of antigen-specific antibodies, APS activates B cells and macrophages, enhances T cell proliferation, and regulates cytokine production [60,61]. As we observed in our study, goats produced IgA, IgG, and IgM in response to supplemented AMP. Pathogenic and nonpathogenic immune challenges may be protected against by these responses. This suggests that the immunomodulatory effect of AMP is primarily due to the polysaccharide fractions. According to Youssef *et al.* [2] the suboptimal supplemented dose of APS did not affect lambs' immune responses, which may be due to the suboptimal supplementation. *A. membranaceus* powder also stimulated the immune system of weaned pigs with its saponin and beta-glucan contents, as shown in Mao *et al.* [62]. The bioactive components of AMP modulate immunity, so it has many beneficial properties.

Several herbs have immunological activity and act as excellent immunostimulants. Microphages and B-cells are activated by APS and saponins [63], antibodies are made, complement is activated, and T lymphocyte proliferation increases, while astragaloside increases T and B lymphocyte proliferation and antibody production [64].

The addition of AMT to feed was found to stimulate serum levels of IgA, IgG, and IgM, especially at the end of the study. IgA, IgG, and IgM are important markers of humoral immunity. With 50 g/kg of DMI AMT supplementation, immune improvement was also observed in lambs [2]. An increase in serum immunoglobulins and a regulation of multiple cytokines are described by Wang *et al.* [65] for the Tibetan sheep diet that included AMT.

As reported by Cui *et al.* [66], a daily dose of AMP up to a level of 20 g/animal had no significant effect on insulin levels.

Effect of *Astragalus membranaceus* supplementation on rumen parameters

There is an optimal pH range between 6.2 and 7.2 for the growth of fibrolytic bacteria, which is inhibited at pH levels below 6.0 [67]. AMT supplementation increased the rumen fluid pH, but the goat's optimal pH was maintained. AMT increased the concentration of rumen TVFA and NH_3-N content, indicating that AMT improved rumen fermentation. According to Deng *et al.* [68], AMT extract enhanced ruminal fermentation and propionic acid production along with ruminal TVFA in steers' in-vitro study.

Moreover, plant extracts and secondary plant metabolites have been shown to improve rumen fermentation through their effects on ruminal microflora [69]. In the rumen, NH_3-N plays a key role in microbial protein synthesis as an intermediate component of protein

metabolism [70]. As demonstrated in a previous study [2], AMT can play a role in enhancing dietary protein degradation and microbial protein synthesis by increasing rumen ammonia concentrations following AMT supplementation.

We also found that *A. membranaceus* root supplementation enhanced the concentrations of volatile fatty acids and ammonia nitrogen in the rumen because our results also showed a promoting effect on rumen parameters. As a result, animals and rumen microflora gained more energy and nitrogen, and eventually grew and gained weight [2].

Conclusion

A. membranaceus root extract improves immune status and antioxidant activity in-vitro and in live animals and has a potent effect on hematology and rumen fermentation. Moreover, it has a potent cytotoxic effect on cancer cells. It is necessary to conduct further research on *A. membranaceus* to achieve maximum body performance, and increase immunity and resistance to illnesses in Egyptian goats.

Abbreviations

AMP, *Astragalus membranaceus* root powder; COX-1, 2, cyclooxygenase enzyme 1, 2; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; MTT, methyl-thiazolyl tetrazolium; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TVFA, total volatile fatty acid.

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Conflicts of interest

There are no conflicts of interest.

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