



Altered Hematological and Selected Serum and Rumen Constituents of Egyptian Rahmani Sheep Fed on Dried Chinese Herbal *Astragalus Membranaceus* Root Extract Supplemented Ration



CrossMark

Alaa H. Jaheen^{1*}, Fady Sayed Youssef², Mohamed E. Ali¹, Mohamed A. El-Sherif¹, and Mohamed I. Oraby¹

¹Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

²Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

A NATURAL herbal product well-known as *Astragalus membranaceus* root that is widely distributed in China; contains many biologically active components that are used for medicinal purposes. This study determined the value of supplementation *Astragalus membranaceus* root powder (AMP) to the daily feed on hematological constituents, rumen parameters, immunoglobulins, and antioxidant response in Egyptian Rahmani sheep. In addition to, the in-vitro anti-inflammatory activity study of AM extract. A total number of twenty-five Rahmani sheep were joined randomly in our study with average weight (60-70 kg) and age (5-6 years old). After 2 weeks of adaptation, sheep received 20 g/ animal/ day of (AMP) mixed with their daily feed for 28 days. Statistical significant increase in hematological components, leukocyte count, catalase activity, total antioxidant capacity, Immunoglobulin A, Immunoglobulin M, and Immunoglobulin G contents after 14 days and 28 days of the daily feed of 20g of AMP that enhanced by time with no difference in insulin level. In contrast, malondialdehyde showed a lower concentration on 14 and 28 days of supplementation with an increase in total volatile fatty acid and rumen ammonia nitrogen content after AMP fed on days 14, and 28 compared to zero days with enrichment in protozoal activity. The in-vitro study showed AMP possesses a potent anti-inflammatory activity.

We concluded that, using AMP as a natural feed additive can enhance hematology and rumen parameters with improvement in immunity and antioxidant activity in Rahmani sheep. Also, it possesses anti-inflammatory activity.

Keywords: Herbal extracts, Rahmani sheep, fermentations, Immune status, Oxidant state.

Introduction

Astragalus membranaceus is one of the most popular medicinal legumes which are used worldwide. It is mainly originated in China and known as “Huangqi.” and is used as medicine and food additive to revitalize the spleen [1]. The *Astragalus membranaceus* root (AMT) contains

many biologically active constituents such as polysaccharides and saponins which have been widely used in traditional medicine for many years as a promoter of immunity [2].

Astragalus polysaccharide (APS) which is derived from stems or dried roots of *A. membranaceus* is a water-soluble

*Corresponding author: Alaa H. Jaheen, Email: dr.alaaahelal@yahoo.com, Tel: 01067648819

(Received 18/06/2023, accepted 12/07/2023)

DOI: 10.21608/EJVS.2023.218519.1527

©2023 National Information and Documentation Center (NIDOC)

heteropolysaccharide that possesses biological functions [3]. It is considered the most vital natural active compound in AM that possess many pharmacological functions [4]. In consequence of its low side effects and biohazards; in addition to non-residual, and non-tolerance functions [5].

Recent studies have shown that AM roots attain antioxidant, anti-inflammatory, along with antiviral and antimicrobial functions with growth-promoting and immunity-enhancement activities as mentioned by some authors [6,7]. Improving immunity and enhancing the synthesis and production of cytokines and immunoglobulin by the active product; Astragalus polysaccharide (APS), as well as having a cytotoxic effect on tumor cells by boosting tumor cell apoptosis and down-regulating cancer cell propagation [1].

Although many research studies had shown that AMT has a lot of pharmacological actions like pain killer and sedative properties, body tonics, and immuno-enhancement, also, hepato-protective function and lowering blood glucose levels [8], besides it can increase growth and strengthen the immune system in pigs [9]. Scarce papers had studied AMT effects on ruminants.

Our research aimed to block this gap by exploring the effects of daily AMT supplementation on hematology and rumen parameters, antioxidant and immune states in Egyptian Rahmani sheep.

Material and Methods

Preparation and preliminary phytochemical testing of Astragalus membranaceus root powder (AMP) extract

Astragalus membranaceus powder (R type) was extracted from dried roots of *Astragalus membranaceus* (Dried Root of high quality from Qi Jing Ltd., Beijing, China) at the Department of Pharmacology, Cairo University in Egypt's Faculty of Veterinary Medicine. Ethanol, CaO, and Na₂CO₃ were obtained from Sigma Aldrich Company. According to the described method by [10]; a simple water extract of AM was evaporated to a predetermined amount, then adding of 100% ethanol allowing the mixture to stand overnight after that, the resultant extract was separated and centrifuged.

Using traditional phytochemical methods, alkaloids, tannins, flavonoids, carbohydrate/glycosides, and resin were all determined in AM extract according to the method described by Pant *et al.*[11].

GC-MS Analysis

A VF-Wax CP 9205 fused silica column (100% polyethylene glycol, 30 m 0.25 mm, 0.25 m) was used for the GC-MS on an Agilent 7890 B gas chromatograph (Agilent Technologies, Rotterdam, The Netherlands). It was connected to an Agilent Technologies mass selective detector 5977A. As previously reported, the instrumental parameters and the separation conditions were used [12].

In vitro anti-inflammatory assay (membrane stabilization method)

Preparation of erythrocyte suspension

Fresh whole blood (3 ml) collected from healthy sheep into heparinized tubes was centrifuged at 3000 rpm for 10 min. An equal volume of normal saline and the supernatant was used for dissolving the pellets of the red blood cells. The dissolved red blood pellets volume was then measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH₂PO₄, 1.15 g of Na₂HPO₄, and 9 g of NaCl in 1 liter of distilled water. The reconstituted red blood cells (resuspended supernatant) were used as such as described by Anosike *et al.*[13].

Hypotonicity-induced hemolysis

The extract samples were dissolved in a hypotonic solution of distilled water in this test. In centrifuge tubes double pairs of 5 ml of the hypotonic solution comprising extracts at different concentrations (100, 200, 400, 600, 800, and 1000 µg/ml) was added. Also, isotonic solution (5 ml) containing the extract at different graded doses (100 – 1000 µg/ml) was put into duplicate pairs (per dose) of the centrifuge tubes. 5 ml of the distilled water (vehicle) and 5 ml of 200 µg/ml of indomethacin respectively were used in control tubes. A suspension of erythrocytes (0.1 ml) was added to each tube and gently mixed. The mixtures were incubated for 1 hr. at room temperature (37°C), and afterward, centrifuged for 3 min at 1300 g. Using Spectronic (Milton Roy) spectrophotometer, the supernatant absorbance (OD) of the hemoglobin content was measured at 540 nm. Then, the hemolysis % was estimated by assuming the hemolysis produced in the presence of distilled water as 100% according to Anosike *et al.*[13]. The hemolysis inhibition % by the extract was calculated thus:

$$\% \text{ Inhibition of hemolysis} = 1 - ((\text{OD2} - \text{OD1}) / (\text{OD3} - \text{OD1})) * 100$$

Where OD1 = sample test absorbance in an isotonic solution

OD2 = absorbance of the test sample in a hypotonic solution

OD3 = absorbance of the control sample in a hypotonic solution

Experimental strategy

The concepts and the methodology for our research were according to the guidelines regulated by the Institutional Animal Care and Use Committee of Cairo University (IACUC) (Ethics approval number: Vet CU 03162023748). A total number of twenty-five adult female Rahmani sheep with average weight (60-70 kg) and ages ranging from 5-6 years old, in the non-pregnant and non-lactating states were used in our study. Rahmani sheep were accommodated in a private sheep farm in El-Fayoum Governorate, Egypt. They fed 450g concentrates and 700g hay as a daily basic feed twice a day where water and trace minerals were freely available. After ten days of adaptation, all sheep joined the study by adding 20g (4.44 percent of concentrate ration) of *Astragalus membranaceus* powder (AMP) per animal as a daily feed intake along with their routine feed for 28 days.

Sampling

On days zero, 14, and 28, before the morning feed, samples of blood were taken from the Jugular vein into plain vacuum tubes, and tubes containing EDTA. EDTA-containing tubes were used for the analysis of hematological parameters such as Red blood cells count (RBCs), hemoglobin (Hb) gm%, platelets count, PCV% along with total leukocyte count (TLC) using Auto-Hematological Analyzer (Mindray BC-2800).

For serum separation, the plain tubes were centrifuged at 3000 rpm for 10 min used for insulin (INS) measurement Insulin ELISA kits (LSBio, WA, USA) at 450nm as stated by manufacturer guidelines. Using ELISA kits of (MyBioSource, USA) superoxide dismutase (SOD), glutathione peroxidase (GSH- PX), total antioxidant capacity (T-AOC), and serum immunoglobulin (IgA, IgG, and IgM) were analyzed, while malondialdehyde (MDA) and catalase (CAT) were analyzed through (BIO-DIAGNOSTIC, Egypt) ELISA kits.

In the early morning on days zero, 14, and 28, rumen fluids samples were sucked with the aid of a stomach tube (Anscitech, Wuhan, China) from each sheep. About 70 ml was sucked from each

sheep where the initial 20 ml was discarded to reduce the contaminated saliva. Using a portable pH meter (913 pH meter; Metrohm, Herisau, Switzerland) the pH was measured. After that, rumen fluid was filtered using four layers of cheesecloth and stored at -80 °C in 10 ml tubes till the examination. Chromatograph (BEIFEN SP-3420A, Beijing, China) was used to determine total volatile fatty acids as detailed by Zhang et al.[14], and the rumen ammonia-N (NH₃-N) as mentioned by Hristov et al.[15].

The tabulated data are expressed as mean ± standard error (mean ± SE). The data were statistically analyzed by using analysis of variance (ANOVA), the difference was considered significant when (P < 0.05) using SPSS 27 (IBM, NY, USA).

Results

Phytochemical screening

According to the results of phytochemical screening, *Astragalus membranaceus* extract contains glycosides, alkaloids, flavonoids, tannins, and also, saponins with total phenols, and total flavonoid contents (190.14 µg of gallic acid equivalent/ g of dry tested sample and 241.5 g of rutin equivalent/ g of dry tested sample, respectively). No resin was detected in the examined sample.

GC-MS Analysis

The chemical composition of *Astragalus membranaceus* extract was tabulated in Table 1.

In vitro anti-inflammatory assay (membrane stabilization method)

An anti-inflammatory assay was performed to investigate the in vitro anti-inflammatory efficacy of the tested AM extract compared to standard at upgraded concentrations (µg/ml) 100, 200, 400, 600, 800 & 1000. The findings as shown in Table 2 and Fig. 1 showed a concentration-dependent inhibition of hemolysis as follows (99.3%, 96.1%, 94.1%, 92.5%, 91.6%, 90.4%) & (99.5%, 95.9%, 93.6%, 92.4%, 90.2%, 89.1%) for standard and AM extract respectively.

Astragalus membranaceus effect on hematological constituents

Our results showed a significant enhancement in RBCs count, Hb %, PCV%, and platelets count along with a significant increase in leukocyte count after a daily intake of 20 g of *Astragalus membranaceus* powder mixed with the basic diet for 28 days; the results showed a

TABLE 1. Chemical composition of *Astragalus membranaceus* extract using GC-MS Analysis

RT	Compound Name	Chemical formula	MW	Area %
51.45	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	1.19
52.83	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	11.48
53.56	Palmitic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	1.36
54.44	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312	11.44
60.17	Octadecanoic Acid, Ethyl Ester	C ₂₀ H ₄₀ O ₂	312	3.72
63.14	2,3-Dihydroxypropyl Palmitate	C ₁₉ H ₃₈ O ₄	330	0.71
64.64	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	281	0.94
66.1	P-Cresol, 2,2'-Methylenebis[6-Tert-Butyl-	C ₂₃ H ₃₂ O ₂	340	0.77
67.01	Hexadecanoic Acid, 3-[(Trimethylsilyl)Oxy]Propyl Ester	C ₂₂ H ₄₆ O ₃ Si	386	9.14
67.55	Glycidol Stearate	C ₂₁ H ₄₀ O ₃	340	5.21
69.05	Docosyl Heptanoate	C ₂₉ H ₅₈ O ₂	438	1.48
72.53	2,2-DIMETHYL-3-OXA-5 α -CHOLESTANE	C ₂₈ H ₅₀ O	402	17.21
74.51	1-S-[(1e)-2-(1h-Indol-3-Yl)-N-(Sulfoxy)Ethanimidoyl]-1-Thiohexopyranose	C ₁₆ H ₁₉ N ₂ O ₉ S ₂	447	1.58
77.72	3-O-(Trimethylsilyl)-5,7,3',4'-Tetra-O-Methylquercetin	C ₂₂ H ₂₆ O ₇ Si	430	13.98
85.56	3-[(Trimethylsilyl)Oxy]Stigmast-5-Ene	C ₃₂ H ₅₈ OSi	486	1.36
88.13	3',4',7-Trimethylquercetin	C ₁₈ H ₁₆ O ₇	344	6.17
94.26	18-Pentatriacontanone	C ₃₅ H ₇₀ O	506	3.79

RT: Standard Retention Time

TABLE 2. Hemolysis Inhibition% of standard compared to A.M extract.

Conc. (μ g/ml)	Hemolysis Inhibition% of standard	Hemolysis Inhibition% of A.M extract
1000	99.3	99.5
800	96.1	95.9
600	94.1	93.6
400	92.5	92.4
200	91.6	90.2
100	90.4	89.1

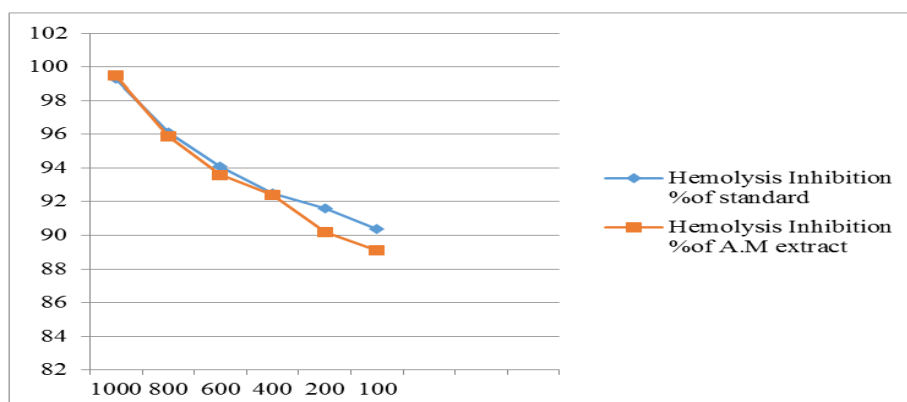
**Fig.1. Hemolysis Inhibition % of standard compared to that of A.M extract showed a concentration-dependent inhibition of hemolysis**

TABLE 3. Hematological constituents of normal Egyptian Rahmani sheep fed on *Astragalus membranaceus* extract supplemented ration at zero, 14 and 28 days of feeding represented by mean \pm SE

Parameters	Rahmani sheep (n=25) Zero-day	Rahmani sheep (n=25) 14 days	Rahmani sheep (n=25) 28 days
Hemoglobin (gm %)	8.4 \pm 0.18 ^a	9.6 \pm 0.3 ^b	10.3 \pm 0.32 ^b
RBCs (10 ⁶ /mm ³)	10.4 \pm 0.24 ^a	12.1 \pm 0.3 ^b	13.1 \pm 0.3 ^c
PCV %	25.02 \pm 0.9 ^a	28.6 \pm 0.8 ^b	30.6 \pm 0.8 ^b
Platelets (10 ³ /mm ³)	473.4 \pm 1.9 ^b	489 \pm 1.14 ^b	521 \pm 1.5 ^a
MCV (fl)	0.35 \pm 0.09 ^a	0.22 \pm 0.1 ^a	0.43 \pm 0.12 ^a
MCH (pg)	23.9 \pm 0.3 ^a	23.6 \pm 0.4 ^a	23.2 \pm 0.38 ^a
MCHC (g %)	8.08 \pm 0.04 ^a	7.94 \pm 0.15 ^a	7.82 \pm 0.09 ^a
WBCs (10 ³ /mm ³)	8.7 \pm 0.6 ^a	8.8 \pm 0.3 ^a	10.06 \pm 0.6 ^a

a: $p \leq 0.001$: highly significant; b: $p \leq 0.01$; c: $p \leq 0.05$; ^{ab} Means within a row with different superscripts differ ($p < 0.05$).

TABLE 4. Serum insulin, selected antioxidant constituents, immunoglobulins, and rumen constituents of normal Egyptian Rahmani sheep at zero, 14 and 28 days of feeding *Astragalus membranaceus* supplemented ration represented by mean \pm SE.

Parameters	Rahmani sheep (n=25) Zero-day	Rahmani sheep (n=25) 14 days	Rahmani sheep (n=25) 28 days
Hormone			
Insulin (ng/ml)	2.5 \pm 0.1	2.5 \pm 0.27	2.5 \pm 0.14
Antioxidant activity			
Superoxide dismutase (mg/dL)	302.57 \pm 1.5 ^b	484.27 \pm 1.7 ^{ab}	498.13 \pm 2.4 ^a
Glutathione peroxidase (mg/dL)	228 \pm 1.01 ^b	348.66 \pm 1.5 ^a	364.3 \pm 1.5 ^a
Catalase (mg/dL)	178.5 \pm 1.9 ^b	227.6 \pm 0.32 ^{ab}	269.9 \pm 0.04 ^a
Malondialdehyde (mg/dL)	5.37 \pm 0.15 ^a	4.29 \pm 0.08 ^b	3.18 \pm 0.04 ^c
Total antioxidant capacity	1.22 \pm 0.096 ^a	1.54 \pm 0.06 ^b	1.83 \pm 0.06 ^c
Immunoglobulins			
Immunoglobulin A(mg/dL)	42.3 \pm 6.5 ^a	53.3 \pm 4.6 ^{ab}	62.3 \pm 3.4 ^b
Immunoglobulin M (mg/dL)	33 \pm 1.15 ^a	54 \pm 1.7 ^b	56 \pm 1.6 ^b
Immunoglobulin G (mg/dL)	276.6 \pm 1.2 ^a	342.3 \pm 2.2 ^b	347.3 \pm 1.5 ^b
Rumen constituents			
pH (g/dL)	6.2 \pm 0.05 ^a	6.5 \pm 0.05 ^b	6.6 \pm 0.03 ^b
Ammonia (g/dL)	12.5 \pm 0.09 ^a	17.4 \pm 0.09 ^b	18.5 \pm 0.03 ^b
Total volatile fatty acid (g/dL)	60.31 \pm 1.7 ^a	67.87 \pm 0.5 ^b	69.72 \pm 0.2 ^b
Rumen protozoa	+	++	+++

a: $p \leq 0.001$: highly significant; b: $p \leq 0.01$; c: $p \leq 0.05$; ^{ab} Means within a row with different superscripts differ ($p < 0.05$)

significant increase after 14 days and 28 days of supplementation which increased by time as tabulated in Table 3.

Antioxidant state

As tabulated in Table 4. Daily supplementation of 20g of AMP enhances SOD activity with a significant improvement in GSH-Px activity in serum. Furthermore, CAT and T-AOC activities were promoted after the feeding of AMP which enhanced with time but the MDA activity significantly decreased on 14 and 28 days of supplementation compared to zero days.

Astragalus membranaceus effect on serum insulin and immunity

The results of serum insulin and immune state are displayed in Table 4. As a consequence of the daily intake of 20 g of AMP/animal for 28 days, IgA, IgM, and IgG contents in serum were increased and promoted on day 14 also, enhanced with time on day 28 with no difference in insulin level compared to zero days.

Astragalus membranaceus impact on rumen components

Compared to zero days, rumen pH was increased on days 14, and 28 as a result of daily AM feed. Moreover, total volatile fatty acid (TVFA) concentration in the rumen was increased significantly on days 14, and 28 compared to zero days with enhancement in rumen NH₃-N concentration production along with an increase in proliferation and activity of normal micro-flora and rumen protozoa as represented in Table 4.

Discussion

Traditional herbal medicine is widely used in China among them; *Astragalus membranaceus* which is a Chinese herbal medicine, that contains many biological active components. Among these active components are polysaccharides which are the most important compound in its root and also contain different amino acids, isoflavonoids, γ -aminobutyric acid, as well as astragaloside [16].

According to the phytochemical screening of the *Astragalus membranaceus* root extract used in our study. The sample was found to contain glycosides, Alkaloids, flavonoids, tannins, and also saponins. As described by Zhang et al.[17] who said that the most important vital composition in AM is polysaccharides, flavonoids, and saponins, together with amino acids, phenols, and sucrose. Polysaccharides which are considered the most vital and biologically active compound in the

roots of AM extract possess a lot of therapeutic functions and effects. Three types of *Astragalus* are present; type I, II, and III according to the type of polysaccharides present in AM. Where type I comprises a carbohydrates chain composed of glucose and arabinose, while *Astragalus* type II comprises a carbohydrates chain composed of glucose, arabinose, and rhamnose. But D-glucose is the only constituent of *Astragalus* type III. Astragaloside is the most predominant polysaccharide in AM. Astragaloside is a steroidal saponin present in *Astragalus membranaceus* which is sub-grouped into 7 groups according to their cycloastragenol structure as described by Zhu et al.[10].

Natural remedies are used to treat illnesses nowadays. Contrarily, synthetic drugs are dangerous and have serious side effects that endanger public health. However, consistent usage of synthetic drugs could lead to serious issues such as the development of drug resistance. Because they have few side effects, herbal remedies are becoming more and more popular [18]. Many therapeutic plants and potential herbs, particularly those with bioactive properties like antimicrobial, antioxidant, anti-diabetic, anti-parasitic, and anti-cancerous capabilities, are still being exposed for improvement to be used in the field of human, animal, and poultry health [19].

Anti-inflammatory activity results agreed with Huang et al.[20] who reported that AM extract reduced the inflammatory response & may effectively prevent the activation of NF- κ B p65 and diminish the expression of cytokines like IL-8 and ICAM-1. In addition, Meng et al. [21], confirmed that AM extract contains polysaccharides that possess potent anti-inflammatory properties.

In addition, Adesso et al.[22] reported that dried root extract of *Astragalus membranaceus* helped reduce intestinal irritation. In the non-tumorigenic intestinal epithelial cell line (IEC-6) tumor necrosis factor (TNF) release, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression, nitrotyrosine formation, nuclear factor-B (NF-B), and reactive oxygen species (ROS) release were all shown to be decreased by the AM extract. The findings emphasized the potential for using AM extract as an anti-inflammatory and antioxidant treatment for intestinal disorders and clarified the processes by which this extract decreased inflammation.

Moreover, Park et al.[23] revealed that the anti-

inflammatory actions of AM extract decreased the expression of TNF-, iNOS, COX-2, and NF-B as well as dramatically inhibited NO generation. To boost the anti-oxidative effect and reduce inflammatory responses, AM extracts can be used as unique and potent natural anti-inflammatory products.

To begin evaluating a new feed additive, we must first assess the health of the animals. The hemato-biochemical contents are excellent markers of the animal's overall health. All blood count parameters were considerably improved compared to zero days by adding *Astragalus membranaceus* powder as a feed additive to the basic meal for 14 and 28 days, indicating that the animals' overall health improved along with the AM dietary treatment.

Supplementing with AMP considerably enhanced most blood counts parameters such as hemoglobin content, RBC count, PCV%, platelet count, and leukocyte count after 14 and 28 days of daily feeding of 20g of AM powder.

Although there are few published papers on the effects of AMP on the erythrogram of sheep, Lv et al. [24] reported that *Astragalus* could reduce bone marrow cell apoptosis and enhance hemopoietic progenitor cell differentiation along with the megakaryocyte and erythroid cell lines. Furthermore, following chemotherapy, anemic mice with *Astragalus* exhibited a considerable rise in the count of colony-forming unit-megakaryocyte (CFU-Meg), indicating that this herb might speed the recovery of megakaryocyte hemopoiesis after bone marrow down-regulation [25]. Furthermore, some researchers, reported that after *Astragalus* addition, there was a large multiplication of colony-forming units of erythroid cells, burst-forming unit-erythroid cells, megakaryocytes, granulocytes, and macrophages[26].

The WBC system is a critical component of the body's defense system, and it is typically recruited from the blood to the site of infection [27] as part of the immune-stimulating system. *Astragalus* appears to have a dual impact: an "immune-enhancing effect" that boosts the generation of B lymphocytes, T lymphocytes, interleukin, and antibodies, as well as an "adaptogenic effect" that exposes viruses, bacteria, and even cancer cells to the immune system [28].

In broilers, *Astragalus* polysaccharides have been shown to increase antioxidant activity and scavenge free radicals at a dietary daily intake

of 5 g/kg [29]. Furthermore, the flavonoids and saponins isolated exhibit antioxidant properties. The essential characteristics for maintaining oxidative state in animals are T-AOC and T-SOD [30].

In our study, AM powder administration raised T-SOD, CAT, and T-AOC activities but decreased MDA levels in the serum, indicating that AM powder improved sheep's antioxidant status. This is congruent with the findings of Zhong et al.[31] who found that dietary AM powder supplementation at 50 g/kg enhanced the activity of T-AOC, T-SOD, and CAT in the serum of weaned lambs. The endogenous antioxidant defense enzymatic system includes glutathione peroxidase, T-SOD, and CAT. As a result, the increased activity of these enzymes following AMP supplementation increased the capacity of sheep to scavenge reactive oxygen species (ROS) and free radicals with a reduction in MDA level, which indicated by lipo-peroxidation reduction.

Furthermore, the increase in serum antioxidant capacity with increasing AMP intake was attributable to the combined action of AMP's antioxidant components. As referred to Kim et al. [32]; AM contains various natural biological ingredients such as polysaccharides, saponins, and flavonoids that have a high antioxidant action. These exogenous antioxidants can boost antioxidant activity by increasing the activity of endogenous antioxidants and scavenging free radicals [33]. Our findings reveal that AMP supplementation has antioxidant benefits in sheep, indicating that AMP might help decrease oxidative stress in sheep during high-stress times in production.

Exogenous antioxidants can reduce oxidative stress by lowering lipid peroxidation and scavenging free radicals, which can then stimulate the immune system. Previous research has shown that AMP has a considerable effect on reducing immunological stress and stimulating the immune system [34]. By raising the formation of antigen-specific antibodies, activating B cells and macrophages, improving T cell proliferation, and modulating cytokine release, *Astragalus* polysaccharide can alter both humoral and cellular immune responses [35]. In our investigation, AMP supplementation influenced sheep immune responses by increasing IgA, IgG, and IgM production. These responses have the potential to protect sheep from both pathogenic and non-pathogenic immunological attacks.

As a result, we think that the immunomodulatory impact of AMP in the current investigation is mostly attributable to the polysaccharide fractions. However, Zhong *et al.* [31] showed that supplementation with *Astragalus* polysaccharide at 15 g/kg food did not affect the immunological responses of lambs, which might be attributed to the inferior supplemented dosage. In addition, Mao *et al.* [36] showed that saponin and beta-glucan in *Astragalus membranaceus* powder have potent immune-stimulating effects in weaned piglets. In other words, AMP contains several bioactive components that have potent immune regulation properties.

Many plants are immunologically active, working as good immunostimulants. *Astragalus* polysaccharides and saponins activate macrophages and B cells, stimulate antibody synthesis, activate complement, and boost T lymphocyte proliferation, whereas astragaloside stimulates T and B lymphocyte proliferation and antibody production [37].

In our investigation, adding AMT as a feed additive had a significant influence on serum IgA, IgG, and IgM concentrations, particularly in the late phases of the trial; IgA, IgG, and IgM are essential indices of humoral immunity. Immune improvement was also observed in lambs after 50 g/kg DMI AMT supplementation [31].

As found by Wang *et al.* [37] the included AMT in the Tibetan sheep diet stimulated the immune system by increasing serum immunoglobulins and regulating the release of a variety of cytokines.

Our investigation found that a daily feed of AMP up to a dosage of 20 g/animal did not influence insulin levels, which was consistent with the findings of Cui *et al.* [38].

A pH of 6.2 to 7.2 is favorable for fibrolytic bacterial development, which is hindered at pH levels lower than 6.0 as stated by Sung *et al.* [39]. Even though AMT supplementation raises the pH of the rumen fluid in sheep, it remains within the optimum range. The concentration of rumen TVFA and ammonia nitrogen content rose with AMT supplementation in the current study, demonstrating that AMT improved rumen fermentation. Similar findings were reported in an *in vitro* research on steers by Deng *et al.* [40], who found that AMT extract increased ruminal fermentation and the formation of rumen TVFA as well as propionic acid. Furthermore, several

plant extracts and secondary plant metabolites impacted the activity of ruminal bacteria, which promoted rumen fermentation as described by Busquet *et al.* [41].

Recent research by Zhong *et al.* [31] demonstrated that AMT can have a role in boosting the breakdown of food protein and the production of microbial protein by increasing the concentration of rumen ammonia. Furthermore, daily intake of AM root extract proved to increase the rumen concentration of volatile fatty acids and ammonia nitrogen, which is considered a source of energy and nitrogen for animals and rumen microflora, eventually encouraging animal growth and weight gain.

Conclusion

Astragalus membranaceus root extract enhances immunological status and antioxidant activity in live animals and has a powerful influence on hematology and rumen fermentation. It also has high anti-inflammatory activity. Further research on *Astragalus membranaceus* in Egyptian Rahmani sheep is required to achieve peak body performance, enhance immunity, and resistance to various illnesses.

Acknowledgments

We are grateful to the Department of Pharmacology where the extraction of AM has worked out, Faculty of Veterinary Medicine, Cairo University, Egypt. Also, to the private sheep farm in El-Fayoum Governorate where the experiment on the animals has been conducted.

Funding Statements

The authors declare that the present study has no financial issues to disclose.

Conflict of interest

None

Authors contributions:

All authors contributed to the study's conception, and design. Data collection, clinical examination and experimental study were performed by MIO, FSY, and AHG. All biochemical analysis and data analysis were performed by MAE, and MEA. MIO, FSY, and AHG drafted and corrected the manuscript; MAE and MEA revised the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

- Zheng, Y., Ren, W., Zhang, L., Zhang, Y., Liu, D. and Liu, Y. A Review of the Pharmacological Action of Astragalus Polysaccharide. *Front. Pharm.*, **11**, 349 (2020). doi: 10.3389/fphar.2020.00349.
- Cho, W.C.S. and Leung, K.N. In viro and in vivo immunomodulating and immunorestorative effects of Astragalus membranaceus. *J. Ethnopharm.*, **113**, 132–141 (2007).
- Li, C., You, P.M., Wang, C.X., He, J.X., Xiao, W.Y. and Guo, Z. Research Progress on the Molecular Mechanism of Antioxidation of Astragalus Polysaccharide. *Journal of Minzu University of China (Natural Sciences Edition)*, **40** (04), 78–82 (2019).
- Li, S.S., Qi, Y.L., Ren, D.D., Qu, D. and Sun, Y.S. Astragalus Membranaceus The Structure Features and Improving Effects of Polysaccharide from on Antibiotic-Associated Diarrhea. *Antibiotics, (Basel)*, **9** (1), 8 (2019). doi: 10.3390/antibiotics9010008.
- Zhang, Z. and Zhu, Y.H. Effect of Astragalus Polysaccharide on Prevention and Control of Viral Diseases in Poultry. *J. Anim. Sci. Vet. Med.*, **29** (04), 42–43+47 (2010). doi: 10.3969/j.issn.1004-6704.2010.04.012
- Nie, X., Shi, B., Ao, Z., Yin, G. and Tao, W. Extraction of the *Astragalus* polysaccharides and its effects on the lymphocytes proliferation. *Journal of Anhui Agricultural University*, **31**, 34–36 (2004).
- Rui, L., Chen, W.C., Wang, W.P., Tian, W.Y. and Zhang, X.G. Antioxidant activity of *Astragalus* polysaccharides and antitumour activity of the polysaccharides and siRNA. *Carbohydrate Polymers*, **82**, 240–244 (2010).
- Huang, W.C., Kuo, K.T., Adebayo, B.O., Lin, Y.K., Wang, C.H., Lee, K.Y., Wang, L.S., Yeh, C.T. and Tsai, J.T. Astragalus polysaccharide (PG2) Ameliorates Cancer Symptom Clusters, as well as Improves Quality of Life in Patients with Metastatic Disease, through Modulation of the Inflammatory Cascade. *Cancers (Basel)*, **11** (8), 1054–1067 (2019). doi: 10.3390/cancers11081054.
- Yin, F.G., Liu, Y.L., Yin, Y.L., Kong, X.F., Li, T.J., Wu, C.Y. and Hou, Y.Q. Dietary supplementation with Astragalus polysaccharide enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets. *Amino Acids*, **37**, 263–270 (2009).
- Zhu, Z.Y., Liu, R.Q., Zhou, F., Li, S.F. and Yuan, J. Extraction and purification of *Astragalus* polysaccharide and its anti-tumor activity. *Mod. Food Sci. Technol.*, **4**, 376–379 (2011).
- Pant, D.R., Pant, N.D., Saru, D.B., Yadav, U.N. and Khanal, D.P. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. *J. Intercul. Ethnopharm.*, **6**(2), 170–176 (2017).
- Mamadaliyeva, N.Z., Abdullaeva, N.S., Rosenau, T., Fakhrutdinova, M., Azimova, S.S. and Bohmdorfer, S. Composition of essential oils from four Apiaceae and Asteraceae species growing in Uzbekistan. *Nat. Prod. Res.*, **32**, 1118–1122 (2018).
- Anosike, C.A., Obidoa, O. and Ezeanyika, L.U.S. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *DARU J. Pharm. Sci.*, **20**, (1) 76 (2012).
- Zhang, Z.G., Xu, D.M., Wang, L., Hao, J.J., Wang, J.F., Zhou, X., Wang, W.W., Qiu, Q., Huang, X.D., Zhou, J.W., Long, R.J., Zhao, F.Q. and Shi, P. Convergent evolution of rumen microbiomes in high-altitude mammals. *Current Biology*, **26**, 1873–1879 (2016).
- Hristov, A.N., Ivan, M., Rode, L.M. and Mcallister, T.A. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. *J. Anim. Sci.*, **79**, 515–524 (2001).
- Ma, X.Q., Shi, Q., Duan, J.A., Dong, T.T.X. and Tsim, K.W.K. Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations. *J. Agricul. Food Chemist.*, **50**, 4861–4866 (2002).

17. Zhang, S., Zhang, T., Teng, K.D., Wang, A.R., Yu, Y.F. and Zhang, H. Effect of *Astragalus* polysaccharide powder injection on the density of microvessels and mast cells in ovalbumin-sensitized rat skin. *Journal of China Agricultural University*, **1**, 67–71(2010).
18. Singh, A., Mishra, A., Chaudhary, R. and Kumar, V. Role of herbal plants in prevention and treatment of parasitic diseases. *J. Sci. Res.*, **64**, 50-58 (2020).
19. Jamil, M., Aleem, M.T., Shaukat, A., Khan, A., Mohsin, M., Rehman, T.U., Abbas, R.Z., Saleemi, M.K., Khatoon, A. and Babar, W. Medicinal Plants as an Alternative to Control Poultry Parasitic Diseases. *Life*, **12**(449), 1-13 (2022). <https://doi.org/10.3390/life12030449>.
20. Huang, W.H., Liao, W.R. and Sun R.X. Astragalus polysaccharide induces the apoptosis of human hepatocellular carcinoma cells by decreasing the expression of Notch1. *Int. J. Mol. Med.*, **38** (2), 551–557 (2016).
21. Meng, X., Wei, M., Wang, D., Qu, X., Zhang, K., Zhang, N. and Li, X. Astragalus polysaccharides protect renal function and affect the TGF-beta/Smad signaling pathway in streptozotocin-induced diabetic rats. *J. Int. Med. Res.*, **48** (5), 300060520903612 (2020). doi: 10.1177/0300060520903612.
22. Adesso, S., Russo, R., Quaroni, A., Autore, G. and Marzocco, S. *Astragalus membranaceus* Extract Attenuates Inflammation and Oxidative Stress in Intestinal Epithelial Cells via NF-κB Activation and Nrf2 Response. *Inter. J. Mol. Sci.*, **19**(3), 800 (2018).
23. Park, H.M., Lee, J.Y., Kim, M.Y., Kang, C.H. and Hwang, H.S. Anti-oxidative and anti-inflammatory activities of *Astragalus membranaceus* fermented by *Lactiplantibacillus plantarum* on LPS-Induced RAW 264.7 cells. *Fermentation*, **7**(4), 252 (2021).
24. Lv, Y., Feng, X. and Zhu, B. Study on effect of *Astragalus membranaceus* injection on hematopoiesis in anemic mice with myelosuppression. *Zhong Yao Cai.*, **28**, 791-793 (2005).
25. Zhu, X. and Zhu, B. Effect of *Astragalus membranaceus* injection on megakaryocyte hematopoiesis in anemic mice. *Hua Xi Yi Ke Da Xue Xue Bao*, **32**, 590-592 (2001).
26. Chen, Y., Zhu, B., Zhang, L., Yan, S. and Li, J. Experimental study of the bone marrow protective effect of a traditional Chinese compound preparation. *Phytother. Res.*, **23**, 823-826 (2009).
27. Ted Valli, V.E. and Jacobs, R.M. Structure, function, and disorders of lymphoid tissue. In: D.J. Weiss and K.J. Wardrop (eds), *Schalm's Veterinary Hematology*, Wiley-Blackwell, NJ, USA, 367–375 (2010).
28. Li, J., Zhong, Y., Li, H., Zhang, N., Ma, W., Cheng, G., Liu, F., Liu, F. and Xu, J. Enhancement of *Astragalus* polysaccharide on the immune responses in pigs inoculated with foot-and-mouth disease virus vaccine. *Int. J. Biol. Macromol.*, **49**, 362-368 (2011).
29. Zhang, G.G., Yang, Z.B., Wang, Y. and Yang, W.R. Effects of *Astragalus membranaceus* root processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poultry Science*, **92**, 178–183 (2013).
30. Wang, Y.Z., Xu, C.L., An, Z.H., Liu, J.X. and Feng, J. Effect of dietary bovine lactoferrin on performance and antioxidant status of piglets. *Anim. Feed Sci. Technol.*, **140**, 326–336 (2008).
31. Zhong, R.Z., Yu, M., Liu, H.W., Sun, H.X., Cao, Y. and Zhou, D.W. Effects of dietary *Astragalus* polysaccharide and *Astragalus membranaceus* root supplementation on growth performance, rumen fermentation, immune responses, and antioxidant status of lambs. *Anim. Feed Sci. Technol.*, **174**, 60-67 (2012).
32. Kim, J.S., Yean, M.H., Lee, E.J., Kim, Y.J. and Kang, S.S. Two new cycloartane saponins from the roots of *Astragalus membranaceus*. *Chem. Pharm. Bull.*, **56**, 105-108 (2008).
33. Yan, H., Xie, Y.P., Sun, S.G., Sun, X., Ren, F., Shi, Q., Wang, S., Zhang, W., Li, X. and Zhang, J. Chemical analysis of *Astragalus mongholicus* polysaccharides and antioxidant activity of the

- polysaccharides. *Carbohydrate Polymers*, **82** (3), 636-340 (2010).
34. Jiang, J., Wu, C., Gao, H., Song, J. and Li, H. Effects of Astragalus polysaccharides on immunologic function of erythrocyte in chickens infected with infectious bursa disease virus. *Vaccine*, **28**, 5614-5616 (2010).
35. Qin, Q., Niu, J., Wang, Z., Xu, W., Qiao, Z. and Gu, Y. *Astragalus membranaceus* extract activates immune response in macrophages via heparanase. *Molecules*, **17**, 7232-7240 (2012).
36. Mao, X.F., Piao, X.S., Lai, C.H., Li, D.F., Xing, J.J. and Shi, B.L. Effects of beta-glucan obtained from the Chinese herb *Astragalus membranaceus* and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs. *J. Anim. Sci.*, **83**, 2775-2782 (2005).
37. Wang, X.J., Ding, L.M., Wei, H.Y., Jiang, C.X., Yan, Q., Hu, C.S. and Degen, A.A. Astragalus membranaceus root supplementation improves average daily gain, rumen fermentation, serum immunity and antioxidant indices of Tibetan sheep. *Animals*, **15**(1), 100061 (2021).
38. Cui, K., Zhang, S., Jiang, X. and Xie, W. Novel synergic antidiabetic effects of *Astragalus* polysaccharides combined with *Crataegus* flavonoids via improvement of islet function and liver metabolism. *Mol. Med. Rep.*, **13**, 4737-4744 (2016).
39. Sung, H.G., Kobayashi, Y., Chang, J., Ha, A. and Hwang, I.H. Low ruminal pH reduces dietary fiber digestion via reduced microbial attachment. *Asian Australasian J. Anim. Sci.*, **20**, 200-207 (2007).
40. Deng, D.J., Wang, J., Ren, L.P., Zhou, Z.M. and Meng, Q.X. The effect of different doses of Astragalus root extract on in vitro rumen fermentation of steam-flaked maize grains used as an only substrate. *J. Anim. Feed Sci.*, **16**, 172-177 (2007).
41. Busquet, M., Calsamigila, S., Ferret, A. and Kamel, C. Plant extracts affect in vitro rumen microbial fermentation. *J. Dairy Sci.*, **89**, 761-771 (2006).

حالة صورة الدم ، معايير الكرش ، حالة المناعة و مضادة الأكسدة في الأغنام الرحمانى المصرية بعد اضافة مستخلص جذور العشب الصينية *Astragalus Membranaceus*

الاء هلال جاهين¹ ، فادى السيد يوسف² ، محمدالسعيد على¹ ، محمد أحمد الشريف¹ و محمد ابراهيم عرابي¹
¹ قسم الباطنة و المعديّة - كلية الطب البيطرى - جامعة القاهرة - مصر.
² قسم الادوية و المستحضرات البيطرية - كلية الطب البيطرى - جامعة القاهرة - مصر.

منتج عشبي طبيعى معروف بجذور *Astragalus Membranaceus* متوفر على نطاق واسع في الصين ، يحتوي على العديد من المكونات النشطة بيولوجيًا التي تستخدم للأغراض الطبية. أجريت هذه الدراسة لمعرفة قيمة إضافة مسحوق جذور *Astragalus Membranaceus* (AMP) إلى العلف اليومي على مكونات الدم، ومعايير الكرش ، وحالة المناعة ، والاستجابة المضادة للأكسدة في الأغنام الرحمانى المصرية. بالإضافة إلى دراسة النشاط المضاد للالتهابات في المختبر لمستخلص AM. تم ضم إجمالي عدد ٢٥ من الأغنام الرحمانى بشكل عشوائى في دراستنا بمتوسط وزن (٦٠-٧٠ ± ٥ كجم) وعمر (٥-٦ سنوات). بعد أسبوعين من التكيف، تلقت الأغنام ٢٠ جم / حيوان / يوم من (AMP) ممزوجة مع علفها اليومي لمدة ٢٨ يومًا. زيادة ذات دلالة إحصائية في مكونات الدم ، وعدد الكريات الدم البيضاء ، ونشاط الكاتلاز ، والقدرة الإجمالية لمضادات الأكسدة، والغلوبولين المناعي A ، والغلوبولين المناعي M ، ومحتويات الغلوبولين المناعي G بعد ١٤ يومًا و ٢٨ يومًا من التغذية اليومية ٢٠ جم من AMP والتي تتحسن بمرور الوقت مع عدم وجود اختلاف في مستوى الأنسولين. في المقابل ، أظهر malondialdehyde تركيزًا أقل في ١٤ و ٢٨ يومًا من المكملات مع زيادة في إجمالي محتوى الأحماض الدهنية المتطايرة ومحتوى أمونيا الكرش بعد تغذية AMP في اليومين ١٤ و ٢٨ مقارنة باليوم صفر. أظهرت الدراسة المختبرية أن AMP يمتلك نشاطًا قويًا مضادًا للالتهابات.

استنتجنا إلى أن استخدام AMP كمادة طبيعية مضافة للأعلاف يمكن أن يعزز مكونات الدم ومعايير الكرش مع تحسين المناعة ونشاط مضادات الأكسدة في الأغنام الرحمانى. كما أنه يمتلك نشاطًا مضادًا للالتهابات.

الكلمات المفتاحية: *Astragalus Membranaceus* ، الأغنام الرحمانى ، مكونات الدم ، الكرش ، الحالة المناعية ، مضادات الأكسدة.