# Physiological, molecular, and immune responses to milk thistle extract administration in goats during peripartum period

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**Background**
Using natural compounds as additives in livestock nutrition could be a new goal in livestock production. Milk thistle extract is rich in bioactive compounds such as silymarin, which act as a strong antioxidant agent.
**Objective**
The current study aimed to investigate the metabolic profile, oxidative statue, and immune response after milk thistle extract administration in goats during the peripartum period.
**Materials and methods**
Multiparous pregnant Egyptian Nubian goats ($n$=16) were allocated into four experimental groups. The first group was kept as the control group. The second group was administrated milk thistle extract (10 g/day), whereas third and fourth groups were administrated 20 and 30 g/day for 4 months, respectively. Blood biochemical parameters were measured using colorimetric and enzyme-linked immunosorbent assay. Gene expressions of antioxidant genes [catalase (CAT), superoxide dismutase (SOD1, SOD2), glutathione peroxidase (GPX1), and peroxiredoxin 2] and transcription factor (nuclear factor erythroid 2 related factor 2) were evaluated using quantitative real-time PCR.
**Results and conclusion**
Biochemical parameters (total protein, glucose, total lipids, total cholesterol, triglycerides, high-density lipoprotein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, creatinine, triiodothyronine, and thyroxine) in plasma of groups administrated with milk thistle extract did not significantly differ compared with the control group. Milk thistle extract at high levels (20 and 30 g/day) significantly increased the level of activity of antioxidant enzymes (SOD, CAT, and GPX), total antioxidant capacity, and total immunoglobulin in cases compared with the control group. Moreover, milk thistle extract (20 or 30 g/day) significantly decreased the level of malondialdehyde (lipid peroxidation biomarker) and tumor necrosis factor alpha (inflammatory biomarker) in cases compared with the control group. The results indicated a significant increase in transcript abundance of CAT, GPX1, and SOD1 mRNA in the three groups administrated with milk thistle extract compared with the control group. However, mRNA expressions of SOD2, peroxiredoxin 2, and nuclear factor erythroid 2 related factor 2 were significantly up-regulated after administration with milk thistle extract at high levels (20 and 30 g/day). Milk thistle extract exerts antioxidant, anti-inflammatory, and immune-modulator effects during pregnancy and lactation in goat and maintained normal physiological functions.

**Keywords:**
antioxidant enzymes, gene expression, goat, milk thistle extract, silymarin, total immunoglobulin

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## Introduction

Herbs are certain plants that contain active biological substances. The use of herbs as additives in livestock nutrition instead of other chemical compounds, for example, antibiotics, could be a new goal in livestock production as a natural consequence of the increased demand of safe products for human consumption [1]. In this regard, milk thistle (*Silybum marianum*) is a herb plant that grows well in north Africa and has been used for centuries with medicinal purposes [2–4]. Milk thistle is a versatile crop that has adapted to the

broadly different soil and environmental conditions throughout all continents [1]. Milk thistle seeds are rich in antioxidant agents such as silymarin [3].

In a recent study, milk thistle was added to the diet of buffalo, and the digestibility was improved when it was used up to 20% of the ration [5]. In addition, milk thistle was used in the ration (200 g/kg) of goats, and the results indicated no negative effect on health and digestion, and there was no difference between the diets containing corn or barley [6]. The use of milk thistle in animal feeding ration could be a supplement of active compounds such as flavonolignans, which enhance milk production and quality in addition to its beneficial effect on animal health and overall productivity [7,8]. Indeed, addition of milk thistle into the diet of Comisana ewes has resulted in increased amount of somatic cells in sheep milk during lactation [9]. Refaie *et al.* [10] indicated that growing rabbits that were fed milk thistle extract (1.5 and 3.0 ml/kg diets) gained greater final live weight and feed conversion ratio than the control and kept the values of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (ALP)] at normal values.

Pregnancy and lactation are physiological processes considered to induce oxidative stress and reactive oxygen species (ROS), causing death of cell [11]. Malondialdehyde (MDA) as a product of lipid peroxidation was increased in pregnant sheep and goat fed on low-quality forage, accompanied with a decrease in the total antioxidant activity [11]. Silymarin has been reported to act as an excellent antioxidant, scavenger for ROS and inhibitor against lipid oxidation, thereby could protect animal cells against ROS malfunctions comprising free radicals such as superoxide radical, hydroxyl radical, and hydrogen peroxide [12]. Therefore, the current study was aimed to investigate the metabolic profile, oxidative statue, and immune response after milk thistle extract (rich in silymarin) supplementation in goats during the peripartum period.

## Materials and methods

### Extraction of milk thistle seed

Milk thistle seeds were obtained from local market and extracted with hot water (double distal water) for 1 h using the Soxhlet apparatus. The extract was filtered and then concentrated under controlled reduced pressure at 40°C to obtain a crude extract. The dried crude extract was stored at −20°C until further use.

### Experimental ration composition

Ingredients (%) of the ration consisted of corn (40%), soybean meal (15%), sunflower meal (23%), wheat bran (17%), molasses (2%), limestone (2%), and sodium chloride (1%). Feed samples contained ~15% of crude protein, 3% of crude fat, 9% of crude fiber, 6% of ash, and 67% of nitrogen-free extract.

### Estrus synchronization and mating

The experiment was carried out in the Sheep and Goat Research Unit, Nubaria Agricultural Experimental Station, National Research Center. The average body weight of goats was ~23±2 kg. The goats were diagnosed using sonar (Dawei Portable Sonar, Dawei Medical (Jiangsu) Corp., Jiangsu, China) before estrus synchronization to confirm they were not pregnant and clear of vaginal and uterine illnesses. All females were estrus synchronized using sponge of progesterone and Pregnant Mare Serum Gonadotropin (PMSG) injection (400 IU). Progestagen sponges with 40 mg of fluorogestone acetate (Ceva, Libourne, France) were inserted into the vaginal canal and remained *in situ* for 12 days. Animals were given an intramuscular injection of PMSG on the day of sponge removal (400 IU, Hipra, S.A., Spain). For a period of 15 days, all females were mated naturally 24 h after receiving the PMSG injection with fertile Nubian bucks. Sonar (3.5 MHz transducer) was used to monitor the pregnant female goats 2 months after mating [13].

### The experimental animals and design

Egyptian Nubian multiparous pregnant goats (3 months pregnant, $n$=16) were selected and divided into four identical groups (four animals each). Animals were fed either the control ration or the control rations and orally administrated with different levels of milk thistle extract. Group 1 was fed control ration only and serves as control. Group 2 was fed on control ration and orally administrated with 10 g/animal/day milk thistle extract. Group 3 was fed control ration and orally administrated with 20 g/animal/day milk thistle extract. Group 4 was fed control ration and orally administrated with 30 g/animal/day milk thistle extract. Goats were housed in soil-surfaced pens, and rations were offered twice a day at 7 am and 4 pm in quantities sufficient to meet their energy and nutrient requirements according to NRC [14] recommendations; animals had free access to clean fresh water. The experiment lasted 4 months.

### Blood biochemical parameters

Blood samples were withdrawn from jugular vein into vacutainer tubes contain heparin. Plasma were separated by centrifuging for 15 min at 4000 rpm

and stored at −20°C for subsequent analysis. Biochemical parameters estimated included glucose, total protein, total lipids, total cholesterol, triglyceride, and high-density lipoprotein (HDL). The measurements of blood ALP, ALT, and AST were performed to estimate the liver function of experimental animals and expressed as U/l. Blood urea and creatinine were performed to estimate the kidney function of experimental animals. All analyses were measured using colorimetric methods according to instructions provided by the manufacturer company (Bio Diagnostic, Giza, Egypt).

#### Antioxidant and oxidative status

The blood samples were assessed for enzymatic antioxidants and nonenzymatic antioxidants, which included catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), and total antioxidant capacity (TAC). Lipid peroxidation product such as MDA was measured and expressed as nmol/ml. All analyses were measured using colorimetric methods according to instructions provided by the manufacturer company (Bio Diagnostic).

#### Blood hormone analysis

The measurement of blood thyroid hormones [triiodothyronine (T3) and thyroxine (T4)] was done using specific enzyme-linked immunosorbent assay (ELISA) test kit (Chemux Bioscience Inc., San Francisco California, USA). The coefficients of intraassay and interassay variations were 5.0 and 13.0%, respectively.

#### Blood total immunoglobulin analysis

The plasma level of total immunoglobulin was measured using specific ELISA test kit (Sunlong Biotech, Zhejiang, China) following the protocol recommended by the manufacturer. The coefficients of intraassay and interassay variations were less than or equal to 10.0 and 12.0%, respectively.

#### Inflammatory biomarker (tumor necrosis factor alpha) analysis

The plasma level of tumor necrosis factor alpha (TNF-α) was measured using goat-specific ELISA test kit (Sunlong Biotech) following the protocol recommended by the manufacturer. The coefficients of intraassay and interassay variations were less than or equal to 10.0 and 12.0%, respectively.

#### Total RNA extraction and purification

The total RNA was extracted from all blood samples using GeneJET RNA purification kit (Thermo Fisher Scientific, Vilnius, Lithuania), following the manufacturer's instructions. To elute RNA, a total volume of 30 µl of nuclease-free water was added to the center of the GeneJET RNA Purification Column membrane and centrifuged for 1 min at 12 000 ×g. To remove DNA present with RNA, 1 µl of DNAse was added to 9 µl of RNA sample and then the total mixture (10 ml) was put in a thermal cycler for 30 min at 37°C. After that, 1 ml of EDTA was added to the mixture. Then, the total mixture (RNA, DNAse, and EDTA) was left to incubate in the thermal cycler for 10 min at 65°C. Finally, DNA was degraded and RNA purity and concentration of all extracted blood samples was evaluated using a NanoDrop 2000C instrument (Thermo Fisher Scientific, Wilmington, Delaware, USA). The values of A260/280 nm ratio of purified blood samples were ranged from 1.9 to 2.1, and RNA was stored in −80°C freezer until cDNA synthesis.

#### cDNA synthesis

The revertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used for reverse-transcription of RNA to cDNA according to manufacturer recommendations. The reaction mixture (4 µl of reaction buffer, 1 µl of oligo dt18 primer, 2 µl of dNTPs, 1 µl of RNase inhibitor, 1 µl of reverse transcriptase enzyme) was added to 11 µl of purified RNA samples, then incubated in PCR thermocycler for 60 min at 42°C, and then continued at 70°C for 5 min.

#### Quantitative real-time PCR

Gene-specific primers were designed from the corresponding gene sequences available in the GenBank database (www.ncbi.nlm.nih.gov) using Primer3 software (http://primer3.wi.mit.edu//), as shown in Table 1. The real-time PCR was done by adding 12.5 µl of SYBR green master mix (Thermo Fisher Scientific), 0.25 µl of forward primer, 0.25 µl of reverse primer, and 5 µl of nuclease-free water. Finally, 2 µl of cDNA of each sample was added to reach a total volume of 20 µl. The real-time PCR was done using a StepOnePlus instrument (Applied Biosystems, Foster City, California, USA). The program of PCR was set as 50°C for 2 min, 95°C done for 10 min, and 40 cycles at 95°C (denaturation) for 15 s, then at 60°C for 1 min (annealing), and finally, extension step at 72°C for 30 s. The data generated was analyzed using delta-delta Ct method, and the relative expression of selected genes including CAT, CuZn-SOD1, Mn-SOD2, GPX1, peroxiredoxins (PRDX2), and nuclear factor erythroid 2-related factor 2 (NFE2L2) were normalized to housekeeping gene (GAPDH).

**Table 1 Primer sequences of genes used for real-time PCR**

| Gene name | Gene bank accession number | Primer sequence | Fragment size (bp) |
|---|---|---|---|
| GPX1 | XM_005695962.3 | F: 5'-AGTTTGGGCATCAGGAAAAC-3' | 178 |
| | | R: 5'-CCGAAGGAAGGCGAAGAG-3' | |
| CuZn-SOD (SOD1) | NM_001285550.1 | F: 5'-TGCAGGCCCTCACTTTAATC-3' | 216 |
| | | R: 5'-CTGCCCAAGTCATCTGGTTT-3' | |
| Mn-SOD (SOD2) | XM_018053428.1 | F: 5'-GTGATCAACTGGGAGAATGT-3' | 163 |
| | | R: 5'-AAGCCACACTCAGAAACACT-3' | |
| CAT | XM_005690077.3 | F: 5'-GAAACGCCTGTGTGAGAAC-3' | 142 |
| | | R :5'-ACATAGGTGTGAACTGCGT-3' | |
| NFE2L2 | XM_013968675.2 | F: 5'-TAAAACAGCAGTGGCTACCT-3' | 159 |
| | | R :5'-GAGACATTCCCGTTTGTAGA-3' | |
| PRDX2 | JN986830.1 | F: 5'-CCAGAAAGTTGTCCAGTGAT-3' R: CAAGTCATTGATGGTGACCT-3' | 116 |
| GAPDH | XM_005680968.3 | F: 5'-AGGTCGGAGTGAACGGATTC-3' | 219 |
| | | R: 5'-GGAAGATGGTGATGGCCTTT-3' | |

bp, base pair; CAT, catalase; GPX, glutathione peroxidase; NFE2L2, nuclear factor erythroid 2 related factor 2; PRDX, peroxiredoxin; SOD, superoxide dismutase.

**Table 2 Blood biochemical parameters of goats administrated with milk thistle extract during pregnancy**

| | Control | Milk thistle extract 10 g/day | Milk thistle extract 20 g/day | Milk thistle extract 30 g/day |
|---|---|---|---|---|
| Glucose (mg/dl) | 99.28a±23.05 | 92.87a±3.00 | 83.77a±16.94 | 84.34a±13.02 |
| Total protein (g/dl) | 6.53a±0.91 | 6.37a±0.12 | 6.74a±0.96 | 6.75a±0.89 |
| AST (U/L) | 9.48a±0.40 | 8.52a±0.30 | 9.36a±1.01 | 9.74a±1.68 |
| ALT (U/L) | 67.79a±2.98 | 75.43a±3.69 | 77.02a±3.05 | 71.67a±3.44 |
| Alkaline phosphatase (μ/l) | 89.03a±4.28 | 88.77a±2.87 | 91.37a±13.82 | 91.27a±15.43 |
| Urea (mg/dl) | 96.30a±12.51 | 121.76a±2.63 | 118.29a±14.27 | 126.85a±17.30 |
| Creatinine (μmol/l) | 95.50a±6.65 | 90.15a±3.61 | 84.80a±8.88 | 88.23a±11.47 |
| Total lipids (mg/dl) | 375.00a±34.85 | 416.82a±12.62 | 425.25a±10.64 | 422.88a±32.36 |
| Total cholesterol (mg/dl) | 173.65a±10.05 | 193.94a±54.83 | 231.87a±39.39 | 252.24a±48.00 |
| Triglyceride (mg/dl) | 15.65a±1.82 | 13.95a±1.14 | 12.14a±0.51 | 15.16a±0.21 |
| HDL (mg/dl) | 32.15a±6.23 | 34.75a±4.39 | 31.06a±5.60 | 34.89a±2.46 |
| T3 (ng/ml) | 1.08a±0.12 | 1.34a±0.40 | 1.46a±0.15 | 1.37a±0.21 |
| T4 (ng/ml) | 6.43a±1.69 | 6.73a±1.07 | 7.60a±1.51 | 7.37a±1.50 |

Values were mean±SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; T3, triiodothyronine; T4, thyroxine. The same latter in same row means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

### Statistical data analysis

The results were expresses as mean and analyzed using one-way analysis of variance followed by Tukey's comparison test using the SPSS statistical program. Differences were considered significant at $P$ value less than or equal to 0.05 [15].

## Results

### Effect of milk thistle administration on kid mortality

The total number of birth of kids was 12, 13, 15, and 13 for group 1, group 2, group 3, and group 4, respectively. The total number of death of kids was three, two, one, and one for group 1, group 2, group 3, and group 4, respectively. The kid mortality percentage was decreased in the groups administrated with milk thistle extract at levels of 10, 20, and 30 g /day (15, 7, and 8%, respectively) compared with the control group (25%).

### Effect of milk thistle administration on goat physiological parameters

The profiles of different physiological parameters such as glucose, total protein, total lipids, cholesterol, triglyceride, and HDL were at normal level and were not different among different groups (Table 2). In addition, the levels of plasma ALP, AST, and ALT (indicators of liver function) were not different among different groups (Table 2). Moreover, plasma urea and creatinine (indicators of kidney function) were at the normal level among different groups (Table 2). The profile of thyroid hormones (T3 and T4) was at a normal level among different groups (Table 2).

**Table 3 Effect of milk thistle extract on inflammation biomarker, lipid peroxidation, antioxidant enzyme activity, and total antioxidant capacity in the plasma of goats**

| | Control | Milk thistle extract 10 g/day | Milk thistle extract 20 g/day | Milk thistle extract 30 g/day |
|---|---|---|---|---|
| Catalase (U/ml) | 1.95a±0.35 | 2.85a±0.07 | 3.35b±0.07 | 3.75b±0.35 |
| SOD (U/ml) | 27.20a±1.84 | 35.25a±3.75 | 39.95b±1.06 | 42.45b±2.05 |
| GPX (μg/ml) | 19.10a±0.85 | 22.70a±1.13 | 23.40b±1.13 | 28.20b±1.56 |
| TAC (mmol/l) | 0.633a±0.12 | 0.850ab±0.07 | 1.050bc±0.07 | 1.167c±0.06 |
| MDA (nmol/ml) | 3.05a±0.35 | 2.35ab±0.06 | 2.20bc±0.13 | 1.50c±0.14 |
| TNF-α (μg/ml) | 4.00a±0.14 | 3.55ab±0.64 | 2.50bc±0.28 | 1.60c±0.14 |

Values were mean±SD. GPX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor alpha. The same latter in same row means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

### Effect of milk thistle administration on goat antioxidant capacity

Antioxidant enzyme (CAT, GPX, and SOD) levels were significantly increased ($p<0.05$) in the two groups administrated with milk thistle extract at 20 and 30 g/day compared with the control group (Table 3). The TAC level was significantly increased ($p<0.01$) in the two groups administrated with milk thistle extract at 20 and 30 g/day compared with the control group (Table 3). CAT, GPX, SOD, and TAC levels were not significantly change after administration of milk thistle extract at level 10 g/day compared with the control (Table 3).

### Effect of milk thistle administration on malondialdehyde level

The plasma level of MDA was decreased significantly in the two groups administrated with milk thistle extract at 20 g/day ($p<0.05$) and 30 g/day ($p<0.01$) compared with the control group (Table 3). MDA level was not significantly change after administration of milk thistle extract at level 10 g/day compared to control (Table 3).

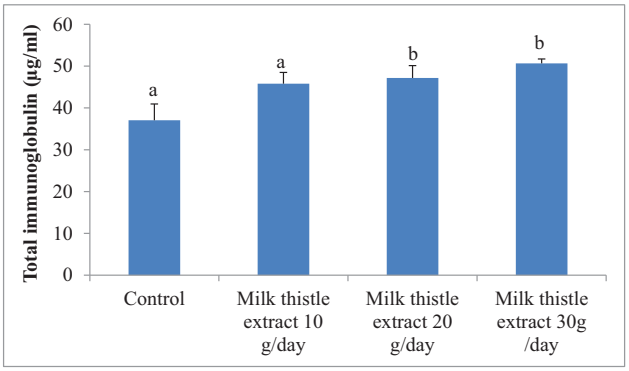
### Effect of milk thistle administration on tumor necrosis factor alpha level

The plasma level of TNF-α was decreased significantly ($p<0.05$) in the two groups administrated with milk thistle extract at 20 g/day ($p<0.05$) and 30 g/day ($p<0.01$) compared with the control group (Table 3). The TNF-α level was not significantly change after administration of milk thistle extract at level 10 g/day compared with control (Table 3).

### Effect of milk thistle administration on total immunoglobulin level

The total immunoglobulin level was significantly increased ($p<0.05$) in the two groups administrated with milk thistle extract at 20 and 30 g/day compared with the control group (Fig. 1). The total immunoglobulin level was not significantly change after administration of milk thistle extract at level 10 g/day compared with the control group.

**Figure 1**




Effect of milk thistle extract on total immunoglobulin level in the plasma of goats. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

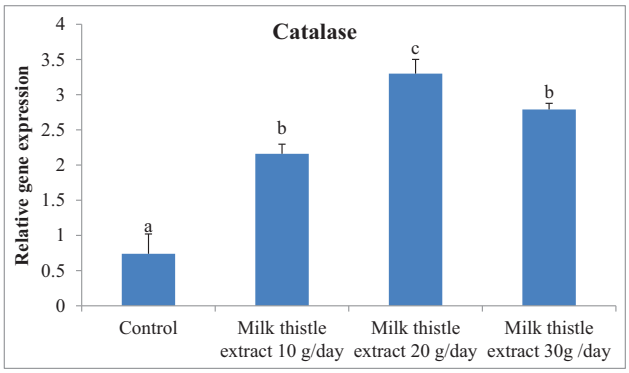
### Effect of milk thistle administration on goat gene expression profile

The expression profiles of CAT, GPX1, and SOD2 were significantly increased ($p<0.05$) in all groups administrated with milk thistle extract at different concentrations (10, 20, and 30 g/animal/day) compared with the control group (Figs 2–4). The expression profile of SOD1 was increased significantly ($p<0.05$) after feeding administrated with milk thistle extract at 20 and 30 g compared with the group administrated with low dose (10 g/day) and the control group (Fig. 5). The expression profiles of NFE2L2 and PRDX2 were significantly increased ($p<0.05$) in the two groups administrated with milk thistle extract at 20 and 30 g/day compared with the control group (Figs 6 and 7).

## Discussion

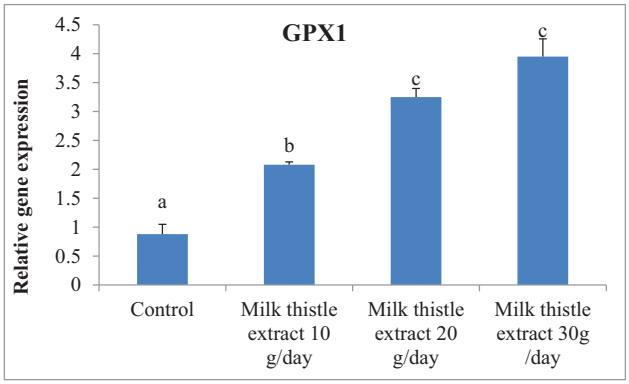
Goats are essential livestock species worldwide owing to excessive adaptability, short growth period, and high

**Figure 2**



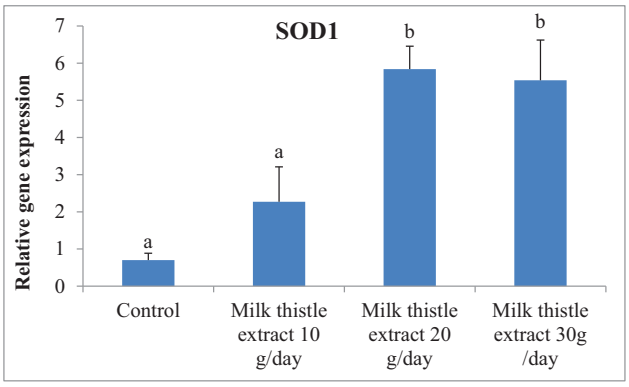

Expression profile of catalase (CAT) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean ±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

**Figure 3**



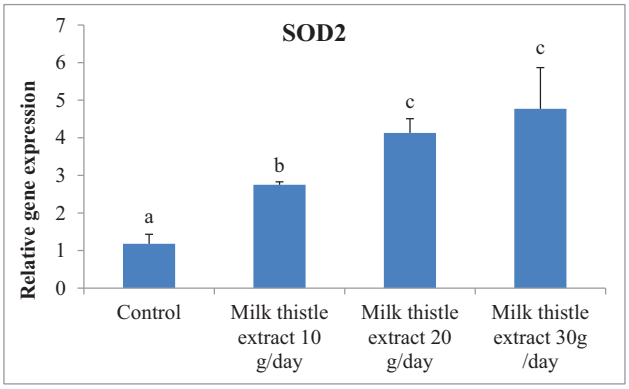

Expression profile of glutathione peroxidase 1 (GPX1) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

**Figure 4**




Expression profile of superoxide dismutase 2 (SOD2) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

**Figure 5**

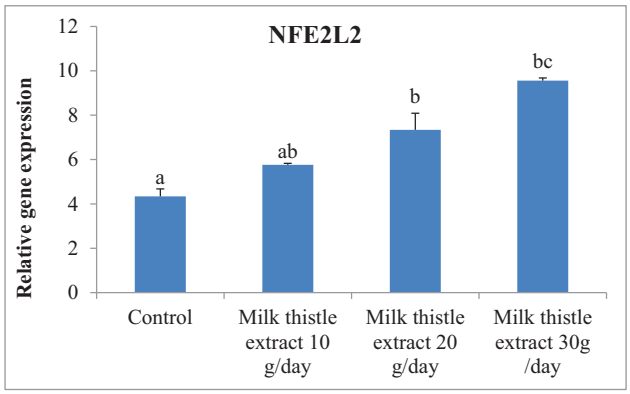



Expression profile of superoxide dismutase 1 (SOD1) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

productivity [16]. Goat production aims to produce high-quality of products companied with special attention to the health of goat. Pregnancy and lactation are physiological process considered to induce oxidative stress owing to increase of oxygen demands to ensure a good growth and development of fetal [17]. Oxidative stress can damage DNA and alter lipids and proteins causing various diseases [18]. A wide range of antioxidant systems protect animals against oxidative stress [19]. Free radicals such as superoxide are catalyzed to hydrogen peroxide by SOD. Hydrogen peroxide is converted into water by CAT, PRDXs, and GPX. Therefore, antioxidant enzymes such as PRDXs, SOD, CAT, and GPX neutralize free radical and reduce oxidative stress [20]. Feeding livestock with rations rich in antioxidant substances such as flavonoids improves the antioxidant status of the animals and reduces oxidative stress damage [21]. Polyphenols including silymarin exert a beneficial effect on health of animals through reducing oxidative stress and inflammation [22]. The present study aimed to investigate the effect of feeding pregnant goats on ration supplemented with silymarin-rich extract from milk thistle to improve its immune and antioxidant status.

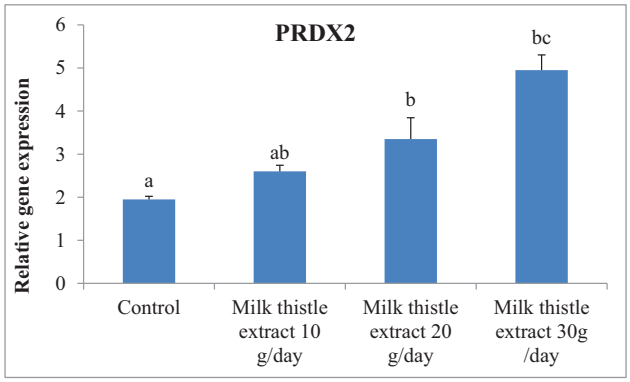
Lipid peroxidation status is an indication for occurring oxidative stress, and high MDA level in blood is an indication of lipid peroxidation induction [23]. Lipid peroxidation status and antioxidant enzyme activity change dramatically in goats during pregnancy and lactation [11,24]. TNF-α, which is an inflammatory cytokine responsible for a various range of signaling events in the cells, induces inflammation and oxidative stress [25]. In the present study, milk thistle extract (20 and 30 g/day) reduced MDA and TNF-α levels in

**Figure 6**




Expression profile of nuclear factor erythroid 2 related factor 2 (NFE2L2) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

**Figure 7**




Expression profile of peroxiredoxins 2 (PRDX2) gene in blood of goats administrated with milk thistle extract during pregnancy. Values were mean±SD, the same latter means nonsignificant difference; different letter mean significant difference at 0.05 probabilities.

blood of cases compared with the control group. Restoration in lipid peroxidation and inflammatory biomarker (TNF-α) in pregnant goats after administration of milk thistle extract could be due to the high content of silymarin in milk thistle extract. Silymarin (flavonolignans compound) has anti-inflammatory effects, acts as a strong antioxidant agent, and has high ability to scavenge ROS [26,27]. The ability of silymarin to scavenging free radical affects GPX and SOD-related enzymes and activates antioxidant enzymes such as CAT, SOD, and GPX [28,29]. There is an increase in the profile of antioxidant enzymes (CAT, SOD, and GPX) in the present study. Milk thistle extract (rich in silymarin) may contribute to increase in the antioxidant enzymes to reduce lipid oxidation.

Interestingly, all physiological parameters of goats that are administrated with milk thistle extract were maintained in normal ranges. Biochemical parameters (total protein, glucose, total lipids, total cholesterol, triglycerides, HDL AST, ALT, ALP, urea, creatinine, T3, and T4 levels in plasma of groups administrated with milk thistle extract did not significantly differ compared with the control group. Similarly, feeding rabbit with ration that had 0.2 or 1% processed *S. marianum* fruits or its extract kept the values of liver enzymes (AST, ALT, or ALP) at normal values [11]. Silymarin has been reported to act as an excellent antioxidant, scavenger for ROS, and inhibitor against lipid oxidation, thereby could protect animal cells against ROS malfunctions comprising free radicals such as superoxide radical, hydroxyl radical, and hydrogen peroxide [12].

NFE2L2 is a protein-coding gene also known as nuclear factor erythroid 2-related factor 2 (NRF2). NFE2L2 is a transcription factor that binds to antioxidant response elements and activates the expression of genes involved in oxidative stress response [30]. Antioxidant pathway is induced by NFE2L2 including CAT, SOD1, GPX, and PRDXs, which are enzymes mediating the elimination of ROS [31,32]. In addition to antioxidant responses, NFE2L2 has an immunomodulatory and anti-inflammation effect [33,34]. NFE2L2 inhibits the transcription of proinflammatory cytokine genes (such as TNF-α and IL-6) and alters immune cell differentiation, expansion, and survival [32]. Nutritional status affects NFE2L2 activity. Ma *et al.* [35] reported that feeding dairy goats a high-grain diet supplemented with sodium butyrate improved antioxidant status through the activation of NFE2L2-related genes. Several *in-vitro* and *in-vivo* studies reported that silymarin acts as a potent inducer of NFE2L2 [36–39]. Goats fed on milk thistle extract rich in silymarin in this study exhibited up-regulation in the expression of NFE2L2 and NFE2L2-regulated genes (CAT, SOD1, SOD2, GPX, and PRDX2) and decreased the level of proinflammatory cytokine (TNF-α), indicating the protective effect of milk thistle extract against harmful effects of oxidative stress and cytokines during pregnancy and the lactating period.Ruminants especially goats have alteration in their immune system during pregnancy [40,41]. Immune system modulation is known to be vital for successful pregnancy. Natural plant secondary metabolites may act as immunological modulators [42]. Many studies suggested the immunomodulatory effect of silymarin from milk

thistle extract in a dose and time-dependent manner [43]. In the present study, feeding goats ration supplemented with milk thistle extract rich in silymarin increased the total immunoglobulin level.

## Conclusion

The use of milk thistle extract as dietary additive in goat rations increased the antioxidant defense through the activation of transcription factor (NFE2L2), induced the expression of the antioxidant enzymes (CAT, SOD, GPX, and PRDXs), reduced MDA level, and therefore protected against oxidative stress, which occurs during physiological stress of pregnancy and lactation. The use of milk thistle extract exerts anti-inflammatory effects and strengthens the immune system and maintains normal physiological functions.

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### Conflicts of interest

There are no conflicts of interest.

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