Evaluation of therapeutic efficacy of medicinal herbal mixture in sheep ruminal acidosis

El-Nady, H.A.1, Abdel-Raof, Y.M1, Ghanem, M.M1, El-Attar, H.E.1, Abd-Elghany, A.H2, Heba M. El-khaliat1
1Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University
2Department of Animal Medicine, Faculty of Veterinary Medicine, Menoufa University

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ABSTRACT

This study was made to assess the therapeutic efficacy of medicinal herbal mixture powder (Cinnamon, Rhubarb, Gentian, Nux Vomica, Nutmeg, Ginger and Boldo) in field cases of sheep with ruminal acidosis. Ten sheep suffering from ruminal acidosis were used to achieve this goal. In addition, ten apparently healthy sheep were used as a control. The sheep suffering from ruminal acidisis showed anorexia, weakness, dullness, pasty feces, tympany and unwilling to move. There was significant (p<0.05) increase in pulse and respiratory rates, while body temperature and ruminal movement were significantly (p<0.05) decreased. There was significant (p<0.05) increase in Hb content, PCV%, RBCs, granulocyte, lymphocyte, and monocyte in acidotic sheep than healthy one. Biochemically, there was significant (p<0.05) decrease in Na, Cl, Mg, Ca, SOD, catalase, albumin and total protein, whereas significant (p<0.05) increase in K, P, AST, ALT, GGT, ALP, urea, creatinine, MDA, CRP, histamine and vitamin B12 in acidotic sheep. The clinical, hematological, biochemical, and ruminal parameters were significantly changed toward the control values after treatment with medicinal herbal mixture. Therefore, these herbal mixtures are recommended as supportive treatment of ruminal acidosis in sheep.

1. INTRODUCTION

Gastrointestinal disorders are of great economic importance among sheep diseases due to their high mortality rate, reduced weight gain and the cost of the treatment (Andrés et al., 2007). Lactic acid results in chemical rumenitis and its absorption causes lactic acidosis. Then, the animal consequently develops hemocoagulation, cardiovascular collapse, renal failure, muscular weakness, shock, and death. Animals that survive may develop mycotic rumenitis in several days, hepatic necrobacillosis several weeks or months later, or chronic laminitis, as well as evidence of ruminal scars at slaughter (Allen et al., 2005). Nowadays, in Egypt there is an increasing trend towards the usage of herbal medicine. These methods have the advantage of using locally available materials which have medicinal properties (Aboelsoud, 2009). The herbal plants extract can be helpful for modification of ruminal fermentation, as feed additives for improvement of animal health status and performance of liver and kidney function and used in treatment of indigestion in sheep and decrease in feed intake (Wafaa, 2017).

Herbal digestive tonic and appetizer is scientifically well verified to maintain a balance between beneficial bacteria and pathogens for intestinal and general health. In addition, facilitates optimal absorption and utilization of nutrients and thus improves feed conversion ratio, productivity, and weight gain (Tiwari et al., 2014).

Nux vomica has antioxidant, anti-inflammatory and pain-relieving effects and is used to treat constipation and other digestive problems (Jon, 2018). Gentian is one of the most important medicines used to treat stomach troubles by the promotion of saliva secretion, acceleration of gastric juice secretion, promotion of viscous liquid secretion, bile secretion and enhancement of stomach motility (Kohlein, 1991). Ginger has antioxidant, anti-inflammatory, anti-tumor, pain-relieving, liver-protecting activities, and antimicrobial effect (Nattha Wannissorn, 2019). Rhubarb improves digestion, stimulates bone growth, prevents neuronal damage, boosts skin health, optimizes metabolism, improves circulation, and protects against various cardiovascular conditions (John Staughton, 2019). Cinnamon has anti-inflammatory properties, it is possible that the consumption of it could reduce both systemic and specific inflammation and has antioxidant benefits (Andrea and Emily, 2018).

Nutmeg treats are used for stomach disorders by helping in getting rid of flatulence, diarrhea, and improves appetite as well. It has detoxifier effect for kidney and liver, as it can remove the toxins. In addition, it is anti-inflammatory and pain reliever properties (Agbogidi and Azagbaekwe, 2013). Boldo has antioxidant, anti-inflammatory, antimicrobial and hepatoprotective effects. So, it can be used in treatment of liver congestion, hepatic insufficiency, and oxidative stress-associated diseases (Mazutti et al., 2008).

This study was designed to evaluate the efficacy of medicinal herbal mixture (Cinnamon, Rhubarb, Gentian,
Nux Vomica, Nutmeg, Ginger and Boldo) on clinical and hematobiocenial alterations which associated with ruminal acidosis in sheep.

2. MATERIAL AND METHODS

2.1. Animals:
Ten field cases of sheep with ruminal acidosis at the age of 1-2 years and weighting 25-55 kg belonging to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University. Another ten healthy sheep were kept as a control.

2.2. Treatment protocol:
Ten affected sheep with lactic acidosis were treated by medicinal herbal mixture powder that obtained from the local market (every 100 gm contain 20 gm Cinnamon, 20 gm Rhubarb, 10 gm Gentian, 10 gm Nux Vomica, 10 gm Nutmeg, 20 gm Ginger and 10 gm Boldo) at a dose of 25 gm per head once daily for 5 days. Additional therapy includes oral antacids such as sodium bicarbonate (ADWIA Co., SAE 10° of Ramadan City) at 1 g/kg b. wt. initially to alkalinize the rumen, and oral electrolyte solutions, preferably those containing additional sodium bicarbonate were used to treat metabolic acidosis. Lavage of the rumen with a wide bore stomach tube in combination with transfusion of ruminal fluid from a healthy animal is preferable. Antibiotics including penicillin, sulphonamide and tetracycline 20 gm orally daily for 3 days to reduce the risk of liver abscessation. Antihistaminic (Avil 1 ampoule intramuscular daily for 3 days) used to reduce adverse effects of histamine release.

2.3. Sampling:
All samples were collected from control healthy sheep and diseased sheep before treatment and on the seventh days post treatment. Two sets of blood samples were obtained from each sheep. Whole blood samples were collected for determination of hematological parameters, while serum samples were collected for biochemical analysis.

2.4. Clinical examination:
Body temperature (°C), and respiratory rates, pulse rates, mucous membrane, and ruminal motility of diseased and control sheep were examined and recorded following the methods described by Kelly (1974).

2.5. Hematological examination:
Complete blood counts including total red blood cell count, hemoglobin (mg/dl) content, PCV%, total white blood cell count and differential leukocytic count (lymphocytes, monocytes, and granulocytes). Hematological examination was done by Hematology Analyzer (XF9080) according to the procedures described by Jain (1993).

2.6. Biochemical examination:
Total protein (g/dL) was determined by colorimetric method by using special kits according to the method that described by Gornall (1949). Albumin was determined by quantitative colorimetric method according to Young (1975). Enzymatic colorimetric test was used for the determination of urea in blood serum using of a special kit according to Eisenwiener (1976). Creatinine was determined by colorimetric test by using of the special kits according to Tanganelli et al. (1982). Calcium, phosphorus, magnesium, sodium, chloride, and potassium levels were determined spectrophotometrically by colorimetric method according to Jansen et al. (1991). Kinetic determination of ALT and AST was measured according to klin (1972). Serum GGT activity was determined calorimetrically according to Beleta and Gella (1990). ALP was determined spectrophotometrically according to Rec. GSCC. DGKC (1972). SOD was determined according to Nishikimi et al. (1972). L-MDA concentration was determined according to Mesbah et al. (2004). Catalase was determined according to Aebi (1984). Histamine was determined according to method described by Stutes et al. (1987). C-reactive protein was determined according to Gerwurz et al. (1982). Vitamin B12 was determined according to Segal et al. (2004).

2.7. Ruminal juice examination:
The ruminal juice was collected from diseased and control sheep by using a stomach tube with a suction syringe 50 ml capacity. Each sample (100 ml) was taken from different level of the ruminal contents in a clean dry and sterile flask. The ruminal fluid was sieved and strained through 2 folds of sterile gauzes and immediately used for estimation of ruminal pH, protozoal activity, motility, and numbers and determine physical characters (color, odor, consistency, and sedimentation activity test). The ruminal samples were preserved for further investigation as methylene blue reduction test (Redistils et al., 2007).

2.8. Statistical analysis:
The data were statistically analyzed using one-way analysis of variance (Bailey, 2008). Values were represented as means ± standard error (SE). All differences were considered significant different when P<0.05.

3. RESULTS

Clinical examination
The affected sheep with ruminal acidosis showed anorexia, weakness, dullness, pasty feces, tympany and unwilling to move. There was significant increase (p<0.05) in respiratory and pulse rates, while the body temperature and ruminal motility were significantly decreased (p<0.05). With advanced cases, the diseased sheep showed dullness, inactive and depression. Pulse and respiratory rate increased, while ruminal motility was completely absent. Diseased sheep showed diarrhea, dyspnea, incoordination and recumbency. The clinical parameters of diseased sheep suffering from acidosis after treatment by medicinal herbs were significantly returned to the normal value (Table1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>Before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>39.15±0.08</td>
<td>38.64±0.07</td>
<td>39.11±0.05*</td>
</tr>
<tr>
<td>Respiratory rate/min</td>
<td>23.75±0.18</td>
<td>30.60±0.43</td>
<td>23.88±0.04*</td>
</tr>
<tr>
<td>Pulse rate / minute</td>
<td>79.60±0.25</td>
<td>92.33±1.22</td>
<td>79.78±0.35</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05

Hematological examination
There was a highly significant increase (p<0.05) in Hb content, PCV%, RBCs, WBCs, granulocyte, monocyte, and lymphocyte in diseased sheep compared to control sheep.
The hematological parameters significantly returned toward the normal values after treatment with medicinal herbs (Table 2).

Table 2 Hematological examination in control sheep and sheep suffering from acidosis before and after medicinal herbal treatment (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10) before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>10.98±0.41*</td>
<td>12.65±0.32</td>
</tr>
<tr>
<td>PCV%</td>
<td>31.0±0.58*</td>
<td>33.07±0.33</td>
</tr>
<tr>
<td>RBCs (10^6)</td>
<td>4.78±0.23*</td>
<td>5.77±0.14</td>
</tr>
<tr>
<td>WBCs (10^3)</td>
<td>12.4±0.40*</td>
<td>18.79±0.56</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.69±0.34*</td>
<td>3.65±0.21</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8.6±0.47*</td>
<td>12.66±0.33</td>
</tr>
<tr>
<td>Monocyte</td>
<td>1.24±0.18*</td>
<td>3.55±0.45</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05.

Biochemical analysis (Tables 3-5)

There was a significant increase (p<0.05) in AST, ALT, GGT, ALP, urea, and creatinine in sheep affected with ruminal acidosis. While albumin and total protein showed significant decrease (p<0.05). There was a highly significant decrease (p<0.05) in serum levels of sodium, chloride, magnesium, and calcium while there was a highly significant increase in serum level of potassium and phosphorus. There was a significant decrease (p<0.05) in serum SOD, Catalase, and vitamin B12 in diseased sheep. While there was a significant increase (p<0.05) in serum MDA, CRP, and Histamine in the affected sheep. These changes returned toward the normal range after treatment with medicinal herbs.

Table 3 Biochemical analysis of sheep suffering from acidosis before and after medicinal herbal treatment (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10) before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/l)</td>
<td>50.0±2.31*</td>
<td>80.8±4.89*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>20.3±1.40*</td>
<td>73.0±4.32*</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>29.7±2.98*</td>
<td>62.7±2.93*</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>23.9±7.01*</td>
<td>343.7±29.07*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.11±0.15*</td>
<td>3.14±0.05*</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.6±0.12*</td>
<td>6.6±0.10*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.25±0.01*</td>
<td>1.1±0.11*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22.1±0.98*</td>
<td>44.9±7.0*</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05.

4. DISCUSSION

Ruminal acidosis is defined as a decrease in the ruminal pH. The reduction in pH below the normal (optimum 6.8) has a significant impact on microbial activity, rumen function, and animal productivity and health (Nagaraja and Lechtenberg, 2007). In the present study, sheep with ruminal acidosis showed decrease feed intake, weakness, depression, semisolid feces, lowered head with slightly distended abdomen. There was significant increase in respiratory rate with labored respiration by stimulation of respiratory centers (Radostits et al., 2014) and Soha (2017). The low pH explains the increased activity of ruminal amylase and amylase, which are toxic enzymes. The observed diarrhea in most sheep could be attributed to the absorbed toxic amides and amines (Mohamed, 2014). The mechanism of rumen stasis could result from the involvement of hydrogen ion receptors elsewhere in the gastro-intestinal tract and or the central inhibition of gastric center by the absorbed toxic amides and amines (Mohamed, 2014).

The observed diarrhea in most sheep could be attributed to conversion of lactic acid to sodium lactate which passed down to the intestine producing an osmotic gradient and compared to control sheep. These changes resumed near the normal values after treatment with medicinal herbs.

Table 5 Biochemical analysis of serum SOD, catalase, MDA, CRP, Histamine and Vitamin B12 in control and diseased sheep before and after medicinal herbal treatment (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10) before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/l)</td>
<td>3.62±0.21*</td>
<td>5.35±0.15*</td>
</tr>
<tr>
<td>Catalase (U/l)</td>
<td>19.46±0.23*</td>
<td>7.41±0.15*</td>
</tr>
<tr>
<td>MDA (U/l)</td>
<td>21.99±1.74*</td>
<td>55.1±1.05*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>3.52±0.23*</td>
<td>14.4±2.08*</td>
</tr>
<tr>
<td>Histamine (U/l)</td>
<td>7.17±0.47*</td>
<td>26.8±5.71*</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05.

Table 6 Physical properties of ruminal juice in control and diseased sheep before and after medicinal herbal treatment (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10) before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Olive green</td>
<td>Yellowish</td>
</tr>
<tr>
<td>Consistency</td>
<td>Slightly viscous</td>
<td>Milky grey</td>
</tr>
<tr>
<td>Odor</td>
<td>Aromatic</td>
<td>Watery</td>
</tr>
<tr>
<td>S.A.T (minutes)</td>
<td>4.5±0.18*</td>
<td>40.8±0.77*</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05.

Table 7 Microscopical examination of ruminal juice in control and diseased sheep before and after medicinal herbal treatment by (Qualitative and Quantitative method) and biochemical analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10) before treatment (n=10)</th>
<th>7th day post-treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoplasts</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Activity Protoplast (x10^3/microl)</td>
<td>4.5±0.15*</td>
<td>1.5±0.12*</td>
</tr>
<tr>
<td>pH</td>
<td>6.7±0.07*</td>
<td>5.8±0.66*</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>2.9±0.17*</td>
<td>6.7±0.33*</td>
</tr>
<tr>
<td>Reduction test/ min.</td>
<td>3.2±0.13*</td>
<td>3.2±0.13*</td>
</tr>
</tbody>
</table>

Means with different letters within the same row differed significantly at p<0.05.
draws water into small intestine contributing to diarrhea (Blood and Radostits, 1989). Excessive lactic acid produced increase ruminal osmolality and water is drawn in from systemic circulation to the rumen causing haemo- concentration and dehydration (Radostits et al., 2007). All these disturbances were attributed to excessive lactic acid production, histamine, methanol and its action on the vital organs and nerve centers and changes in the pH of the rumen, (Radostits et al., 2007). Hematological examination revealed a highly significant increase in Hb concentration, PCV %, WBCs, RBCs, granulocyte, lymphocyte, and monocyte. Our results were matched with those obtained by Garry (2002), Noura (2012) and Soha (2017). The significant increase in erythrocytes, leukocytes and PCV % confirmed that a degree of haemo- concentration and dehydration occurred which increased with the amount of fluids withdrawn from the extracellular fluid space into the rumen. The gastro- intestinal disorders during ruminal acidosis was aggravated, influencing the Leukocytes count toward an increase (Radostits et al., 2007). Biochemically, there was a significant increase of AST and ALT activities which agreed with the results obtained by Patra et al. (1996) and Soha (2017). This increase in transaminases (AST and ALT) may result from blood acidosis and lesions which occurred in the liver due to lactic acidosis (Bradford, 1990). The significant increase in AST 24 hours after induction of acidosis related to liver and muscle damages, the significant increase of ALT activity could be resulted from hepatobiliary injury related to ruminal acidosis (Patra et al, 1996). In addition, there was significant increase in the activity of GGT which coincided with the results obtained by Bionaz et al. (2007) and significant increase in ALP activity which was like the results obtained by Ghanem et al. (2008). Total protein and albumin were significant decreased in sheep suffered from acidosis as obtained by Jorge and Enemark (2008). This could be due to the excretion of these parameters with diarrhea (Cao et al, 1987). There was significant increase in urea and creatinine level, this indicate decreased glomulerial filtration rate in sheep with acidosis, renal damage or reduction in effective renal flow and fall in the arterial blood pressure which results in subnormal function as reported by Xu and Ding (2011) and Zain El-Din (2013). Regarding the serum minerals and electrolyte changes, there were a significant decrease in serum magnesium levels, calcium, chloride, and sodium, while potassium level was significantly increased. These results agreed with Jorg and Enemark (2008). The decreased in serum chloride and sodium may be due to the shift of these electrolytes by osmolality from the blood to hypertonic rumen (due to high lactic acid increase hypertonicity in rumen) or due to their losses (Cl and Na) in lactic acidosis associated with diarrhea (Jorg and Enemark, 2008). Hyperkalemia may result from haemo-concentration and dehydration. The decrease in calcium level might be due to a temporary malabsorption due damaged mucosa of intestine (Radostits et al., 2007). Serum levels of SOD and Catalase was significantly decreased in diseased sheep. These results coincided with that obtained by Zain El-Din (2013). While, Serum MDA in diseased sheep was There a highly significantly increased. Deger et al. (2008) explained that the higher MDA concentration in plasma of sheep with gastrointestinal disorders suggests increased production of lipid peroxidation in the liver, and indirectly pointed to enhanced free radical generation. C-reactive protein is acute phase reactant protein that is produced by hepatocytes and has been associated with inflammation and cellular injury and rise rapidly within the first 6 to 8 hours. When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity Vermeire et al. (2005). Our study showed significant increase in serum levels of CRP in acidic sheep that the same results obtained by Danscher et al. (2015). Histamine is an important regulator of feed and water intake in ruminants Rossi et al. (1998). Our data revealed significant increase in serum histamine level in acidic sheep that matched with results that obtained by Piafiez et al. (2008). Cattle and sheep are herbivores and eat plants like grass, which is free of B12. These ruminants have stomachs that contain various microorganisms, including B12-synthesizing bacteria. The B12 synthesized in the rumen is absorbed in the intestine, transferred into the blood and stored in the liver and muscles of the animal or secreted into the milk Watanabe and Bito (2018). Thus our study found significant decrease in serum levels of Vitamin B12 in diseased sheep with lactic acidosis that could be resulting from destruction of B12-synthesizing bacteria in the rumen lactic acidosis due to fall ruminal pH as a result of increase lactic acid production. With regard to the ruminal fluid analysis of acidic sheep, color varied from yellowish to milky grey color, with sour odor, watery in consistency and Sedimentation activity test (SAT) showed significant increase that matched with Duffield (2004) and Mohamed (2014). The milky-grey color of ruminal fluid in diseased cases arisen from excessive feeding in naturally affected cases and sucrose in induced cases and from the watery consistency and sour odor was due to excessive lactic acid production (Radostits et al., 2007). SAT is used as guide to detect the activity of ruminal microflora (Kimberling, 1988). Physical properties of ruminal fluid after medicinal herbal mixture treatment was improved in which color became olive green or yellowish brown, the odor became aromatic, the consistency become slightly viscous and the SAT time was shorter than before treatment. The results were matched with Mohamed (2014) and Soha (2017). The decreased ruminal pH and increase level of lactic acid may result in Death of ruminal microflora as it accustoms the life in neutral media 6.2-7.2 (Steen 2001). These results returned toward the control values after treatment of sheep by medicinal herbal mixture. A highly significant decrease in rumen pH was found in sheep suffering from ruminal acidosis and Methylene blue reduction test took longer time than normal. The decreased rumen pH in the sheep suffering from ruminal acidosis was due to increased production of lactic acid. Methylene blue reduction test used as guide to examine the activity of microflora. The result was matched with Basnir et al. (2015). Our results revealed that clinical, hematological and biochemical changes associated with lactic acidosis in sheep were improved after treatment of the diseased animals with herbal mixture extracts (contain Cinnamon, Rhubarb, Gentian, Nux Vomica, Nutmeg, Ginger and Boldo) that indicate the useful role of these extract to relief lactic acidosis. This result could be due to antioxidant activity,

5. CONCLUSION

Ruminal acidosis in sheep is associated with abnormal changes in the clinical, hematological, biochemical, and ruminal fluid parameters. The use of medicinal herbal mixture (Cinnamon, Rhubarb, Gentian, Nux Vomica, Nutmeg, Ginger and Boldo) significantly restored these changes toward the control values. Therefore, the use of these medicinal herbal mixtures could be recommended as a supportive treatment of ruminal acidosis in sheep.

6. REFERENCES