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Green Tea as a Supportive Treatment for Respiratory Disorders in Calves

Ali M. Metwally, Ibrahim I. Elshahawy, Zaid M. Abubaker

Department of Animal Medicine, Faculty of Veterinary Medicine, Alexandria University

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Correspondence to:
Ibrahim Elshahawy, Hemas_82@yahoo.com

Abstract

Ninety Friesian calves belonging to Alexandria governorates, Egypt, included in this study and classified into 3 groups, group A: Consist of 20 apparently clinically healthy calves kept as a control, group B: contains 35 calves shows respiratory manifestation, treated with traditional therapy, group C: includes 35 calves exhibit signs of respiratory affection, treated with the same traditional drugs in addition to green tea extract (GTE). Complete early rapid recovery observed in group C than traditionally treated calves. Significant changes in serum creatinine and activity of AST and ALT were observed in group B and C in comparison with healthy calves. There were a significant decreases in the mean values of total antioxidant capacity, catalase activity, reduced glutathione (GSH), glutathione peroxidase (GSH-Px) activity and super oxide dismutase (SOD) activity in group B in comparing with control animals and group (C), while the same previously mentioned values showed a significant increase in group C in comparing with group A and B. On the other hand there is a significant increase and significant decrease in the activity of lipid peroxidase in group B and C, respectively when comparing with control group and with each other. The investigation revealed a significant decrease in the mean values of sodium and chloride with significant increase in potassium level in group B when compared with both control calves and group (C), while the same previously mentioned elements showed non-significant changes in group C when compared with the other two groups.

1. INTRODUCTION

Clinical signs of respiratory disease in a group of calves can be very variable, the clinical signs includes: elevated rectal temperature (above 39.6°C), increased respiratory rate, increased respiratory effort, frequent coughing, muco-purulent ocular/nasal discharge, lethargy, loss of appetite and lying down more than usual (NADIS, 2014). (Lykkesfeldt and Svendsen, 2007) found imbalance between lipid peroxides and antioxidants in pneumonia and suggested that, this factor may contribute to the damage of pulmonary endothelium. Therefore, this damage will produce poor perfusion in pulmonary tissue which may induce free radical processes and the inception of free radical peroxidation (Chilvers, et al., 1989). (Lockwood et al., 1994) reported that the results of trials done at 9 different clinics in Europe and USA showed that Florfenicol is more effective than oxytetracycline and amoxicillin in the treatment of acute bovine respiratory system diseases. Flunixin has anti-inflammatory, anti-pyretic and analgesic effects; this molecule is commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature (Weingarten, 2009). Tea (Camellia sinensis) is mainly produced as four varieties; white (made from very young tea leaves or buds), green (made from mature unfermented leaves), Oolong (from partially fermented leaves) and black (from fully fermented leaves)

(Jigisha et al., 2012). Among the health benefits of green tea are: as an antioxidant, anti-inflammatory, anti-carcinogenic, in cardiovascular health, oral health, and as an antimicrobial. Antioxidant effects come from the ability of green tea to limit the amount of free radicals by binding to reactive oxygen species (ROS) (Serafini et al., 2011; Jigisha et al., 2012 and Gupta et al., 2014). Green tea has been shown to have antimicrobial effects against a variety of gram positive and gram negative bacteria (e.g., Escherichia coli, Salmonella spp., Staphylococcus aureus, Enterococcus spp.), some fungi (e.g., Candida albicans), and a variety of viruses (e.g., HIV, herpes simplex, influenza) (Steinmann et al., 2013). Green tea is characterized by its high flavonoid content (polyphenols), mainly catechins (20-30% of the dry weight). The major catechins are epigallocatechin gallate (EGCG), epigallocatechin (ECG), epicatechin gallate (ECG), gallocatechin (GC), and catechin (C). EGCG is regarded as the most important tea catechin because of its high content, corresponding to approximately 40% of the total catechins (Wang et al., 2000). EGCG has antioxidant activities based on free radicals scavenging and metal chelation. Flavonoids (and their fraction-catechins) are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic et al., 2009). In addition to direct antimicrobial effects of catechins including (damage to the bacterial cell membrane, inhibition of fatty acid synthesis, inhibition of enzyme activity, etc.), there are also some effects that may contribute to the total antimicrobial effect; These effects include inhibition of inflammation (particularly inflammation caused by oxidative stress, such as vascular), more specifically, by increasing the synthesis of nitric oxide (Yamakuchi et al., 2008). The objective of the present study was to investigate the effect of green tea extract and traditional therapy (Florfenicol, Flunixin meglumine and Diphenhydramine hydrochloride) on treatment of respiratory manifestation in weaned calves regarding to rapidity of response, serum oxidant/ antioxidant profile, biochemical parameters, serum electrolytes.

2. MATERIALS AND METHODS

Animals

Ninety Friesian calves belonging to Alexandria governorates, Egypt, aged 3 to 6 months and their weights ranged from 60-130 kg, Calves were clinically examined and classified into 3 groups: group (A) consist of 20 apparently healthy calves not show any diseased condition not take any treatment kept as a control calves, groups (B) consist of 35 calves show respiratory manifestation, treated with traditional therapy including Flunixin meglumine (1.1-2.2 mg/kg body weight) intravenous or intramuscular daily for three days, Florfenicol (20 mg/kg body weight) intramuscular three doses with 48 hours intervals, and subcutaneous injection of Diphenhydramine hydrochloride 20 mg as antihistaminic (1 mg/kg body weight) daily for consecutive three days, finally group (C) consist of 35 calves exhibit signs of respiratory affection, treated with previous traditional drugs with oral administration of one litre of green tea extract every 12 hours for 7 days.

Green Tea Extract

Green tea extracts were prepared from china green tea (Al-najea tea – Al-masa company) and extracted according to the method described by (Yen and Chen, 1994), briefly (50 gm) of tea was extracted with boiled water (one litre) for five minutes.

Samples

Blood samples were collected in test tubes from jugular vein without anticoagulant for separation of the serum. Clear serum was transferred into clean, dry, sterile vials and were kept at -20 C° until used.

Serum Biochemical Analysis

Serum total protein, albumin, and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), creatinine were determined using kits supplied by (Bio-labo, France) according to method described by (Mathieu and Sfbc, 1976; Murray, 1984; Doumas and Biggs, 1972; Curtis et al., 1995 and Young, 1995), respectively. Serum Globulin was calculated by subtracting albumin from serum total protein content. While analysis of BUN, Total bilirubin were carried out by using commercial test kits supplied by (Vitro Scient, Egypt) according to method described by (Rock et al., 1987 and Balistreri and Shaw, 1987) respectively.

Estimation of oxidant / antioxidant profiles

The following markers were analyzed using test kits supplied commercially by (Bio-Diagnostic, Cairo, Egypt)

Total antioxidant capacity (TAC) were determined according to (Koracevic et al., 2001); Catalase determined according to (Aebi, 1994); MDA measured by method of (Ruiz-Larrea et al., 1994); level of reduced Glutathione measured according to (Beutler et al., 1963); Glutathione peroxidase (GSH-P_X) was estimated according to by (Paglia and

Valentine, 1967) and SOD was estimated according to (Nishikimi et al., 1972).

Estimation of serum electrolytes

Sodium and potassium values were estimated by using of atomic absorption spectrophotometer according to (Perkin-Elmer, 1967), while chloride value was estimated by the titrimetric method of (Schales and Schales, 1941).

Statistical Analysis

Statistical analysis performed by using computer program statistical package for social science (SPSS, 2008). Results are expressed by mean ± SEM and all the comparisons were done by ANOVA method and considered different when $p < 0.01$.

3. RESULTS

Results of clinical examination of biochemical observation of normal and respiratory manifested calves could be summarized as following:

Groups A (apparently clinically healthy calves):

Physical and clinical examination of calves in group A revealed good appetite, good vitality and performance, normal body temperature (38.8-39.3^{o}C), normal pulse and respiratory rates 66-80/min and 20-30/min, respectively, normal respiration (abdominal respiration) and normal lung sound (normal vesicular sound).

Groups B and C (calves show different respiratory manifestations):

Their clinical observation revealed loss of condition, rough coat, inappetence to anorexia, depression, dry to moist cough, difficult respiration, congested ocular mucous membrane, some calves show bilateral nasal discharge, abnormal vesicular sound and dyspnea, also there are acceleration of respiratory rate and pulse rate with increasing temperature in severely affected than normal one, some of cases suffering from diarrhea besides respiratory manifestation. In traditionally treated group (B) the response to treatment observed later than observed in group C and their calves not returned to its complete recovery, calves not regain the complete appetite. Abnormal rales and nasal discharge not completely disappeared and this reflected on general health condition, weight gain and appearance of calves. In contrary the calves treated with traditional drugs with GTE showed complete response to therapy started after the first 48 hours from first treatment, appetite not significantly altered, nasal discharges and abnormal rales disappeared , temperature, pulse and respiration returned to its original state as in normal calves with maintenance of shiny appearance and good general health condition.

The results of serum biochemical parameters of total protein, albumin, globulin and total bilirubin reveals non-significant changes among different groups, while significant $P \leq 0.01$ changes in the activity of AST and ALT in group B and C in comparison with group A. (Table1).

Table (1): Serum biochemical parameters of total protein, albumin, globulin, ALT, AST, and total bilirubin in group A, B and C. (Mean± SE)

Parameter	Group A	Group B	Group C
	Mean± SE	Mean± SE	Mean± SE
Total protein (g/dl)	7.21±0.14	7.40±0.32	7.93±0.21
Albumin (g/dl)	2.63±0.09	2.59±0.08	2.55±0.19
Globulin (g/dl)	4.58±0.09	4.54±0.08	5.5±0.08
SGPT(ALT) Iu/L	29.14±1.34*	34.37±0.01*	25.76±2.26*
SGOT(AST) (Iu/L)	75.1±1.60*	89.3±8.49*	68.37±8.50*
Total bilirubin (mg/dl)	0.35±0.15	0.39±0.03	0.31±0.03

*Significant at P values ≤ 0.01

AS shown in table (2), there were no significant changes in the values of blood urea nitrogen among group A, B and C. while significant $P \leq 0.01$ increase in the values of serum creatinine were recorded in both treated group in comparison with control one. On other hand this value in group C (treated with traditional therapy and GTE) was slightly decreased than in group B (treated only with traditional treatment).

Table (2): Mean± SE values of blood urea nitrogen and creatinine among group A, B and C.

Parameter	Group A	Group B	Group C
	Mean± SE	Mean± SE	Mean± SE
BUN (mg/dI)	25.06±1.84	28.3±1.73	26.5±1.63
Creatinine (mg/dI)	1.17±0.12*	1.4±0.07*	1.23±0.11*

*Significant at P values ≤ 0.01

As shown in table (3), there were a significant $P \leq 0.01$ decreases in the mean values of total antioxidant capacity, catalase activity, reduced glutathione, glutathione peroxidase activity and super oxide dismutase activity in group B (treated only by known traditional treatment) in comparing with control group (A) and group (C) (treated with both traditional therapy and green tea extracts), while the same previously mentioned values showed a significant ($P \leq 0.01$) increase in group C in comparing with group A and B.

On the other hand there is a significant ($P \leq 0.01$) increase and significant ($P \leq 0.01$) decrease in the activity of lipid peroxidase in group B and C, respectively when comparing with control group and with each other.

Table (3): Oxidant and antioxidant profile among different groups (Mean± SEM)

Parameter	Group A	Group B	Group C
	Mean± SE	Mean± SE	Mean± SE
Total antioxidant capacity (mmol/ml)	1.55±0.11*	0.59±0.08*	2.34±0.18*
Catalase (U/L)	2246.7±70.7*	1484.2±86.3*	3032.8±125.9*
Lipid Peroxidase (MDA) (mmol/ml)	1.94±0.052*	2.58±0.19*	1.59±0.08*
Reduced Glutathione (mmol/ml)	7.86±0.22*	4.69±0.17*	9.06±0.087*
Glutathione Peroxidase (U/L)	201.1±1.74*	174.8±3.34*	214.4±2.88*
Super oxide dismutase (SOD) (U/ml)	317.4±8.89*	269.30±7.08*	408.5±15.15*

*Significant at P values ≤ 0.01

Regarding to the concentration of some serum electrolytes including sodium, potassium and chloride in different groups under investigation, table (4). Showed that, there were a significant ($P \leq 0.01$) decreases in the mean values of sodium and chloride with significant ($P \leq 0.01$) increase in potassium level in group B (treated with traditional treatment for respiratory dysfunction alone) when compared with both control group (A) and group (C) (treated with traditional and GTE), while the same previously mentioned elements showed non-significant changes in group C when compared with the other two groups.

Table (4): Mean (± SE) values of serum electrolytes (Na, K and Cl) in investigated groups.

Parameter	Group A	Group B	Group C
	Mean± SE	Mean± SE	Mean± SE
Sodium (Na) (mEq/l)	141.8±3.08*	125.8±6.85*	136.0±1.61
Potassium (mEq/l)	3.95±0.36*	5.67±2.15*	4.12±0.38
Chloride (mEq/l)	106.2±2.29*	92.4±7.08*	102.2±1.63

*Significant at P values ≤ 0.01

4. DISCUSSION

Veterinarians recommend Anti-inflammatory drugs in conjunction with antibiotics to help in reducing fever and improve first treatment success rates. Recent research with pre-weaned calves demonstrated a significant reduction in clinical signs, respiratory rate and clinical index score when calves were treated with an antibiotic and flunixin meglumine, (Guzel et al., 2010). Several animal studies suggest that green tea has antioxidant effect, antibacterial and antiviral activity (MacKay and Blumberg, 2000).

Most common signs of respiratory diseases observed in this work were consistent with those observed by (Mansour, 2006; Radostitis et al., 2007; Smith, 2015 El-Sebaie et al., 2002; El-Sheikh et al., 1997; Andrews, 2004 and Vestweber, 1999) which attributed to hypercapnia that increase respiratory rate and polypnea occurred, excessive formation and accumulation of ammonia especially in bad ventilated houses, which leads to irritation and inflammation of mucous membranes causing nasal discharge, exudation of inflammatory fluids in respiratory passages and alveolar lumen causing abnormal rales.

Calves of group C which treated by traditional therapy with GTE extract shows characteristic improvement (after 48 hrs. from first administration) in their abnormal respiratory signs (decrease the amount of nasal discharge, lowering cough with recovered respiratory rales). Also calves not suffered from deteriorated appetite and attitude from the first dose of injected drugs with addition of GTE and high percent of recovery with good general health condition, in contrary to that occurred in group B, these results were also observed by (Bateman et al., 1990), calves that have to be treated for respiratory disorders, even if treated successfully, may not gain as well as calves that never need treatment these observed results may be returned to antimicrobial effect of green tea extracts with act synergistically with used medical therapy, in addition to the antioxidative role of GTE that prevent the extension of further inflammation in respiratory passages. Several animal studies suggest that green tea has antioxidant effect, antibacterial and antiviral activity, cancer chemo preventive properties, contributes to a reduction in the risk of cardiovascular disease, enhances weight loss, amongst other effects (MacKay and Blumberg, 2000).

As shown from our results, non-significant changes in the values of total protein, albumin, globulin and total bilirubin were recorded in both group B and C, the

opposite of these results were obtained by (Shereen and Doaa, 2013) these may be attributed to the difference in treatment period which was long period in their study in contrary with our study, while our obtained results agree with same authors whose recorded significant decrease in serum activity AST and ALT in group C (handled with GTE) this may be due to protection of the liver membrane (cytosol) from oxidation by GTE, so prevent leakage of these enzymes into the circulation. Green tea catechins protect the brain, liver and kidney from lipid peroxidation injury (Lin et al., 1998).

Concerning to serum creatinine level in groups under investigation, there were a significant increase in groups A and C when comparing with control one. By observation the level of serum creatinine is slightly decreased (1.23±0.11) in group C than in group B (1.4±0.07) which is treated with traditional therapy only. Administration of green tea extract normalized the levels of plasma creatinine and uric acid (Elshater et al., 2008). Green tea catechins protect the brain, liver and kidney from lipid peroxidation injury (Lin et al., 1998).

Regarding to antioxidant and oxidant profile in calves suffering from respiratory disorders and treated with different therapy. Traditionally treated calves showed significant decrease in total antioxidant capacity, catalase, reduced glutathione, glutathione peroxidase and super oxide dismutase, while the marker of oxidative stress (lipid peroxidase) showed significant increase in this group when comparing with control calves and group C, the opposite results were detected in group C when comparing with control and group B. Green tea is a popular nutraceutical as an antioxidant, antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, an imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Halliwell and Gutteridge, 1985). Catechins are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system (Abdel-Raheim et al., 2009). In vivo studies showed that green tea catechins increase total plasma antioxidant activity (Yokozawa et al., 2002). Green tea polyphenol supplementation in animal models of oxidative stress has also been shown to increase activities of antioxidant enzymes, specifically glutathione peroxidase, and increase concentrations of glutathione (Ramesh et al., 2009). These results disagree with that observed by (Basu et al., 2013) which reported that green tea supplementation showed no effects in activities of glutathione peroxidase or catalase. In contrary obtained data were coincides with that recorded by (Skrzydlewska et al., 2002), intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta; these enzymes are implicated in cellular protection against reactive oxygen species (Yokozawa et al., 1999). Existing data about MDA is agree with that previously obtained by (Yokozawa et al., 2002 and Yokozawa et al., 1999), malondialdehyde (MDA), a marker of oxidative stress, was decreased after green tea intake. These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect. Catechins could protect low density lipoprotein against peroxidation (Yokozawa et al., 2002). These explain the beneficial effect of GTE in our study on enhancement of liver and kidney function with subsequent their effective role in rapid recovery of calves affected with respiratory disorders.

Finally, serum sodium and chloride were significantly decreased with significant increase in potassium level in traditionally treated group (B), these results come in agreement with (El-Sebaie et al. 2002); Mansour, 2006 and Abdullah et al., 2013), while the same elements were not significantly changed in GTE handled group (C). Hyponatremia and hypochloremia in group B may be go back to that respiratory disorders have adverse effect on electrolytes, body fluids and acid base balance. Hyponatremia may results from false hyperglycemia caused by stressful effect of respiratory diseases which may leads to gluconeogenesis. The decrease of sodium and chloride level with an increase of potassium level attributed to respiratory acidosis which occur due to retention of Co2 in the blood (hypercapnia) of pneumonic calves .This manifested biochemically by hyponatremia, hypochloremia with hyperkalemia as a compensatory mechanism for respiratory acidosis (Radostitis et al., 2000). Non-significant changes in the values of serum sodium, potassium and chloride in group C, could be attributed to green tea polyphenols which act as antioxidant can maintain normal body fluids balance, normal electrolytes concentration and normal acid base balance, this indicate that, these polyphenols have beneficial effect in case of dehydration, electrolyte abnormalities and acid base disturbances.

5. Conclusion and recommendation

administration of green tea as supportive agent in respiratory disorders in calves beside traditional therapy has effective role in rapid recovery without deterioration of the affected animals, these returned to antimicrobial and antioxidative role of green tea extract which protect respiratory tract tissue from further inflammation with enhancement of liver and kidney function beside maintenance of body fluid, electrolyte and acid base balance. Further study needed to evaluate the effect of green tea alone in repairing and recovery of animals from diseases.

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