



Clinicopathological changes in calves with respiratory diseases after treatment with essential volatile oil and other drugs

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ABSTRACT

This study was performed on fifteen newly born calves, 3-5-month-old with clinical signs of bronchopneumonia in addition to five clinically healthy calves which considered as control group. After bacterial isolation and identification of the causative microorganisms, fifteen calves from which was found infected with *Pasteurella* spp. and *Haemophilus* spp. The diseased calves were divided randomly into three equal groups which respectively, treated with: Gp.1: were treated dose of draxxin (2.5 mg/kg body weight) by s/c injection in the neck; Gp.2: were received Draxxin and Meloxicam (single I/M injection of 0.5 mg/kg b.wt.), while Gp.3: were received draxxin (single s/c injection of 20 mg/kg b.wt.) +Symopro on drinking water (150-300 ml/1000 L for 5 days). Blood and serum samples were collected from all calves just before treatment, 3 and 10 days post treatment. pH, PO₂ and SO₂ results recorded significant increase in all treated groups. Meanwhile, PCO₂, tCO₂ and HCO₃ results showed significant decrease in all treated groups compared with diseased group before treatment. Haptoglobin and fibrinogen results showed significant decrease in all treated groups compared with diseased group before treatment. GSH-PX results revealed significant increase. Meanwhile, MDA results showed significant decrease in all treated groups which comparatively with those before treatment. There was significant increase in cellular immunity (phagocytic index and phagocytic%) in treated group with draxxin plus sympro when compared with diseased group before treatment.

Keywords: Non-steroidal anti-inflammatory drug, Bovine respiratory diseases, Sympro, Malondialdehyde, Glutathione peroxidase.

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1. INTRODUCTION

Bovine respiratory disease (BRD) constitutes one of the key health issues and most costly problems occurring in cattle in all part of the world. This disease syndrome was originally termed “shipping fever” since signs often occur shortly after arrival in the feedlot (Urban –Chmiel and Grooms, 2012). About 91 percent of calves diagnosed with BRD

were diagnosed within the first 27 days after arrival (Buhman et al., 2000).

Conventional treatment for BRD usually consists of antimicrobial therapy with the application of mucolytics and bronchodilators (Smith, 1996). As the inflammatory response that occurs in BRD forms a significant part of the disease process in the early, and especially chronic, stage, non-steroidal (NSAIDs) anti-inflammatory drugs have been recommended (Lekeux, 1996).

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The essential oils can constitute a powerful tool to reduce the development and dissemination of antimicrobial resistance (Toroglu, 2011). In fact, plant essential oils have been used for many years as pharmaceuticals in alternative medicine and as natural therapy (Abd El-Motaal et al., 2008). Eucalyptus essential oil is effective for treatment a number of respiratory problems. This oil is anti-bacterial, antifungal, anti-inflammatory and decongestant which makes it a good ingredient in many medicines for treating respiratory problems (Sadlon and Lamson, 2010).

This study aimed to determine the effect of Meloxicam (NSAID) in a combination therapy with antibiotic (Draxxin), Antibiotic alone or in a combination of essential volatile oil (Symopro) on calves suffering from naturally occurring bronchopneumonia. Blood gases and some inflammatory, anti-oxidants markers and lastly, cellular immunity was also estimated.

2. MATERIAL AND METHODS

2.1. Experimental Animals:

Twenty newly born calves, up to 3-month-old belonging to a private farm in Sharkia province, from December till February were used in this study. Five of these animals were apparently healthy and free from any internal or external parasites and kept as a control group. The remaining calves were suffering from bronchopneumonia (fever over 40 °C, cough, nasal discharge from mucous, mucopurulent to purulent, dyspnea and anorexia). The first group were treated with long acting Draxxin; the second group were received Draxxin and Meloxicam (NSAID) while, the third group were received Draxxin and Symopro.

2.2. Drugs:

2.2.1. Draxxin®

Tulathromycin (Pfizer-Company) contain 100mg/ml tulathromycin in propylene glycol/water injectable solution ready for use. The recommended dose is a single dose of 2.5 mg/kg bwt by s/c injection in beef and non-lactating dairy cattle (Skogerboe et al., 2005).

2.2.2. Meloxicam (Metacam)®

Solution for injection contains 20 mg/ml meloxicam produced by Boehringer IngelheimVetmedica Company, Germany. The recommended therapeutic dose is a single intramuscular injection at a dose of 0.5 mg/kg of body weight (Friton et al., 2005).

2.2.3. Symopro®

Essential volatile oil on drinking water bottle contain 150-300 ml/ 1000 L for 5 consecutive days (Maxima Company). It is a mixture of eucalyptus, lemon oil, cornment oil, rosemary and Echinacea purpurea (Sadlon and Lamson, 2010).

2.3. Isolation and identification of bacteria:

Nasal swap samples were collected following the antisepsis procedure to prevent possible bacterial contamination risk and placed in transport mediums (Eurotubo collection swab, Delta lab, Spain). Swab sample were cultured in 5% sheep blood agar and MacConkey Agar. Plates were inoculated and incubated aerobically and an aerobically at 37°C for 24-72 hours. Bacteria were identified (Ozkanlar et al., 2012). Streptococcus spp. and Staphylococcus spp. growing in the media were examined in terms of colony structure, Gram staining, microscopic appearance.

2.4. Blood samples:

Blood samples were collected from the jugular vein of each animal just before treatment, 3 and 10-day post treatment.

Sample (1) 2ml of blood was collected in tube containing heparin (50 i.u/ml) used for blood gases, plasma fibrinogen estimation and lastly cellular immunity evaluation. Sample (2) was 3 ml of blood taken without anticoagulant collected in a clean and dry tube and left to clot at room temperature for about 2 hours and centrifuged at 3000 rpm for 15 min. Serum samples were drawn in dry clean capped tubes and kept in deep freeze at – 20 oC for Glutathione peroxidase, Malondialdehyde and haptoglobin estimation.

I- Analysis of blood gases:

Blood gases was determined in whole blood samples by an automated blood gas analyzer using respiratory cassettes (Gastat fax) according to the manufacturer instructions. Right after obtaining the fresh blood samples in a syringe with heparin, the syringe sample were attached to calibrated cassettes into the analyzer.

II- Anti-oxidants concentration:

Serum concentration of Malondialdehyde was measured according to Satoh (1978).

Serum activity of Glutathione peroxidase (GSH-PX) was measured according to Paglia and Valentine (1967).

III- Acute phase proteins:

Plasma fibrinogen concentrations was measured according to a previously described method (Becker *et al.*, 1984).

Serum haptoglobin was determined photometrically as described by Makimura and Suzuki (1982).

2.5. Statistical analysis:

Statistical analysis was performed using the statistical software package SPSS for windows (Version 18.0; SPSS Inc., Chicago, IL). The significance of differences between the experimental groups were

evaluated by one-way analysis of variance (ANOVA) (Kinnear and Gray, 2006).

3. RESULTS

3.1. Results of bacteriological examination:

The bacteriological examination of the cultured swabs from the diseased animals revealed that the isolated bacterial pathogens were *Pasteurella* spp., *Haemophilus* spp. and *Klebsiella* (Table 1).

3.2. Blood gases results:

Results of diseased calves showed significant decrease of pH, PO₂ and SO₂ together with significant increase in PCO₂, HCO₃ and tCO₂ levels. Regarding to blood gases results of the treated groups with draxxin, draxxin plus meloxicam, draxxin plus sympro revealed that pH, PO₂ and SO₂% were significantly increased, PCO₂ results revealed significant decrease. While, tCO₃ and HCO₃ results there were significant decrease at the 3rd day but pH, PCO₂, PO₂, tCO₂, HCO₃ and SO₂% values recorded significant improvement at 10th day when compared with diseased group before treatment (Tables 3&4).

3.3. Acute phase protein and anti-oxidant parameters results:

Diseased calves suffering from pneumonia revealed significant increase in haptoglobin and fibrinogen. Treated groups with draxxin alone, draxxin plus meloxicam, draxxin plus sympro exhibited significant decrease in haptoglobin and fibrinogen in all treated groups when compared with diseased group before treatment. Concerning to the oxidative status of diseased calves, GSH-PX values recorded significant decrease. Meanwhile, MDA values showed significant increase. Treated groups with draxxin alone, draxxin plus meloxicam, draxxin plus sympro revealed significant increase in

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GSH-PX values and significant decrease in MDA values when compared with diseased group before treatment (Table 5&6).

3.4. Immunity results:

In regard to cellular immunity (phagocytic index and phagocytic %) of diseased calves revealed significant

decrease. Meanwhile, treated groups with draxxin alone, draxxin plus meloxicam, draxxin plus sympro revealed significant increase in both (phagocytic index and phagocytic%) in comparison with diseased group before treatment (Tables 6&7).

Table (1): Prevalence of the isolated microorganisms from the nasopharyngeal swabs of the pneumonic cattle-calves: (Total number of the examined animals = 20)

Isolated bacteria	<i>E.coli</i>	<i>Pasteurella</i> spp	<i>Haemophilus</i> spp.	<i>Staph. aureus</i>	<i>Strept. pyogens</i>	Mixed infection	Total No.
No. of positive animals	2	8	4	1	1	4	20
%	10	40	20	5	5	20	100

Table (2) Blood gases of healthy and diseased calves before and 3 days post treatment (n=5)

Groups Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
pH	7.47 ± 0.002 ^a	7.22 ± 0.004 ^d	7.41 ± 0.006 ^c	7.42 ± 0.003 ^c	7.45 ± 0.007 ^b
PCO ₂ mmhg	40.15 ± 0.61 ^d	51.54 ± 0.24 ^a	46.55 ± 0.13 ^b	46.93 ± 0.11 ^b	43.75 ± 0.25 ^c
PO ₂ mmhg	33.72 ± 0.008 ^a	27.46 ± 0.26 ^c	32.55 ± 0.05 ^b	32.56 ± 0.04 ^b	33.49 ± 0.05 ^a
tCO ₂ mmhg	28.58 ± 0.02 ^c	32.38 ± 0.35 ^a	29.90 ± 0.18 ^b	29.30 ± 0.32 ^{b,c}	28.71 ± 0.21 ^c
HCO ₃ mmhg	27.27 ± 0.03 ^d	30.80 ± 0.24 ^a	29.36 ± 0.04 ^b	29.25 ± 0.06 ^b	28.13 ± 0.03 ^c
SO ₂ %	65.14 ± 0.03 ^a	61.88 ± 0.06 ^d	61.77 ± 0.12 ^d	62.85 ± 0.32 ^c	64.77 ± 0.12 ^b

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c) within the same rows indicate significant differences at $P \leq 0.05$.

Table (3): Blood gases of healthy and diseased calves before and 10 days post treatment (n=5)

Parameters	Groups Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
pH	7.46 ± 0.002 ^a	7.22 ± 0.004 ^c	7.43 ± 0.005 ^b	7.44 ± 0.006 ^b	7.46 ± 0.003 ^a
PCO ₂ <i>mmhg</i>	41.17 ± 0.62 ^c	51.54 ± 0.24 ^a	44.55 ± 0.13 ^b	43.83 ± 0.50 ^b	42.07 ± 0.28 ^c
PO ₂ <i>mmhg</i>	33.42 ± 0.004 ^a	27.46 ± 0.26 ^c	32.85 ± 0.05 ^b	32.88 ± 0.05 ^b	33.41 ± 0.04 ^a
tCO ₂ <i>mmhg</i>	28.59 ± 0.02 ^c	32.38 ± 0.35 ^a	29.34 ± 0.18 ^b	29.20 ± 0.05 ^b	28.85 ± 0.25 ^{b,c}
HCO ₃ <i>mmhg</i>	27.28 ± 0.04 ^c	30.80 ± 0.24 ^a	28.35 ± 0.04 ^b	28.56 ± 0.34 ^b	28.21 ± 0.04 ^b
SO ₂ %	65.11 ± 0.04 ^a	61.88 ± 0.06 ^b	63.16 ± 0.91 ^{a,b}	63.31 ± 3.20 ^{a,b}	64.12 ± 0.13 ^a

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c) within the same rows indicate significant differences at $P \leq 0.05$.

Table (4): Inflammatory markers and Oxidative stress of healthy and diseased calves before and 3 days post treatment (n=5)

Parameters	Groups Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
Haptoglobin <i>mg/dl</i>	1.20 ± 0.02 ^e	6.30 ± 0.3 ^a	4.60 ± 0.08 ^b	1.80 ± 0.03 ^d	2.4 ± 0.05 ^c
Fibrinogen <i>mg/dl</i>	510.00 ± 1.87 ^e	839.00 ± 4.39 ^a	750.20 ± 4.03 ^b	633.40 ± 3.20 ^d	736.20 ± 4.587 ^c
GSH-PX <i>ug/hb</i>	13.65 ± 0.25 ^a	4.42 ± 0.30 ^e	8.03 ± 0.14 ^d	9.12 ± 0.26 ^c	10.66 ± 0.11 ^b
MDA <i>mmol/l</i>	29.41 ± 0.04 ^d	41.42 ± 0.53 ^a	39.03 ± 0.47 ^b	35.12 ± 0.61 ^c	30.66 ± 1.11 ^d

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

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Table (5): Inflammatory markers and Oxidative stress of healthy and diseased calves before and 10 days post treatment (n=5)

Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
Haptoglobin <i>mg/dl</i>	1.10 ± 0.03 ^e	6.3 ± 0.3 ^a	3.4 ± 0.06 ^b	1.4 ± 0.02 ^d	2.2 ± 0.07 ^c
Fibrinogen <i>mg/dl</i>	507.80 ± 3.23 ^d	839.00 ± 4.39 ^a	599.60 ± 11.66 ^b	523.60 ± 3.14 ^{c,d}	540.00 ± 5.68 ^c
GSH-PX <i>ug/hb</i>	13.05 ± 0.28 ^a	4.42 ± 0.3 ^c	8.87 ± 0.31 ^d	10.14 ± 0.27 ^c	11.37 ± 0.23 ^b
MDA <i>mmol/l</i>	29.39 ± 0.42 ^c	41.42 ± 0.53 ^a	36.87 ± 0.42 ^b	31.37 ± 0.83 ^c	30.14 ± 0.71 ^c

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

Table (6): Cellular immunity of healthy and diseased calves before and 3 days post treatment (n=5)

Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
Phagocytic index	4.88 ± 0.09 ^a	3.28 ± 0.07 ^d	3.38 ± 0.07 ^d	3.88 ± 0.04 ^c	4.32 ± 0.09 ^b
Phagocytic %	74.00 ± 0.32 ^a	58.40 ± 0.93 ^d	59.20 ± 0.58 ^d	67.40 ± 0.51 ^c	69.20 ± 0.37 ^b

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

Table (7): Cellular immunity of healthy and diseased calves before and 10 days post treatment (n=5)

Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Sympro
Phagocytic index	4.88 ± 0.09 ^a	3.28 ± 0.07 ^d	3.38 ± 0.07 ^d	3.88 ± 0.04 ^c	4.32 ± 0.09 ^b
Phagocytic %	74.00 ± 0.32 ^a	58.40 ± 0.93 ^d	59.20 ± 0.58 ^d	67.40 ± 0.51 ^c	69.20 ± 0.37 ^b

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

4. DISCUSSION

The bacteriological examination of the cultured swabs from the diseased animals revealed that the isolated bacterial pathogens were *Pasteurella* spp., *Haemophilus* spp. and *Klebsiella*. These results are in agreement with Saeed and Emad (1990) and Ismail (1991).

Regarding to blood gases blood pH is a biochemical indicator which must be between narrow intervals (7.2-7.6) for the tissue to perform in the body and affect the body functions negatively and even causes death (Karademir *et al.*, 2001). Results of diseased calves showed significant decrease of pH, PO₂ and SO₂ together with significant increase in PCO₂, HCO₃ and tCO₂ levels these results agree with Cambier *et al.*, (2002) who reported that, blood oxygen levels binding in hypoxic calves PO₂ were investigated they showed that diseased animals exhibited a significant acidosis in arterial and venous blood. Also, our results of decrease in pH supported by (Cambier *et al.*, 2002 and Ok *et al.*, 2005). The lung helps regulate pH in the body by exhaling carbon dioxide and in severe acidosis, there is rapid respiration in an effort to exhale more CO₂ in an effort to correct the imbalance (Montgomery, 2009). In addition, the changes in blood gases were significantly associated with a high respiratory frequency suggesting that the breath rhythm and intensity were insufficient to eliminate CO₂ and supply O₂. Consequently, a respiratory acidosis has occurred, evidenced throughout a significant decrease in the blood pH and a significant increase in the circulating proton concentrations. On the contrary, the plasma bicarbonate concentrations were markedly increased in BRD affected calves, confirming the insufficient CO₂ elimination (Ozkanlaret *al.*, 2012). Similar results were

also reported by Nagy *et al.* (2006) who reported marked hypoxemia and hypercapnia were observed in the calves with severe respiratory disease.

Regarding to blood gases results of the treated groups with draxxin, draxxin plus meloxicam, draxxin plus sympro revealed that pH and PO₂ and SO₂% there were significantly increased. PCO₂ results revealed significant decrease. While, tCO₂ and HCO₃ results showed significant decrease at the 3rd day. pH, PCO₂, PO₂, tCO₂, HCO₃ and SO₂% values recorded significant improvement at 10th day. Our results in group treated with draxxin agree with Tanritanir *et al.* (2010) who reported that compared to control and diseased calves there were significant increase of PCO₂ and HCO₃ levels together with significant decrease in pH and PO₂ but one week after treatment with draxxin, pH, PCO₂ and HCO₃ levels were significantly changed. While, in group treated with draxxin plus meloxicam our findings agree with Lekeux (1996) who reported that acute inflammatory component of pneumonia results in impaired gas exchange and the aim of modulating pulmonary inflammation by the use of NSAIDs are to block the production and/or the effects of inflammatory mediators and modulators which have a deleterious effect on alveolar exchange of gases. But more prominence improvement in group treated with draxxin plus sympro. Similar results obtained by Sadlon and Lamson (2010) who recorded improvement in PCO₂ and SO₂ due to the effect of sympro in relief effect of colds, influenza, other respiratory infections, rhinitis and sinusitis by decrease airway mucin secretion of tracheal and bronchiole epithelium (Wu, 2016 and Nordqvist, 2017).

Diseased calves suffering from pneumonia revealed significant increase in haptoglobin and fibrinogen. Our results are paralleled to the finding of Angen *et al.*

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(2009) who reported that the haptoglobin might be the best choice for detecting respiratory diseases under field conditions and to distinguish between calves requiring anti-inflammatory drug.

Treated groups with draxxin alone, draxxin plus meloxicam, draxxin plus sympro exhibited significant decrease in haptoglobin and fibrinogen in all treated groups which were more pronounced decrease in group treated with draxxin plus meloxicam. Similar results agree with Lynch et al. (2010) and Richeson et al. (2013).

Concerning to the oxidative status of diseased calves, GSH-PX values decreased. Meanwhile, MDA values showed significant increase. These results attributed to decreased antioxidant enzyme activity and reduction of blood GSH-PX concentration and these result from a massive and durable production of radical oxygen species during broncho-pneumonia development and lead to exacerbation of oxidative damage in lung (Al-qudah, 2009). Our results of decrease in GSH-PX in diseased calves with pneumonia agree with (Al-qudah, 2009 and Eleiwa et al., 2014). As it well known that inflammatory diseases are associated with enhanced oxidative reactions and reduce antioxidant defense capabilities (Behiman et al., 2001). These results may be due to the excessive lipid peroxidation in plasma and cells ascribed to many factors or diseases lead to excessive formation of NADPH which in turn promotes lipid peroxidation in the presence of cytochrome P-450 system (Jain, 1989 and Ahmed et al., 2010). Malondialdehyde.MDA is a major and stable end product formed of peroxidation is regarded as a marker of lipid peroxidation (Del et al., 2005).

Treated groups with draxxin, draxxin plus meloxicam revealed that GSH-PX

results revealed significant increase in all treated group. Meanwhile, MDA results showed significant decrease in all treated groups when compared to diseased group. This attributed to the anti-inflammatory effects of meloxicam are limited to eicosanoids that act as pro-inflammatory mediators, including thromboxane, prostaglandins and Malondialdehyde (MDA), while activating early immune mediators such as leukotrienes which are responsible for the stimulation of neutrophil function, through the lipooxygenase pathway (Friton et al., 2005). Treated group with draxxin plus sympro revealed that GSH-PX results showed significant increase compared to diseased group. Meanwhile, MDA results showed significant decrease compared to diseased group. Our results supported by Sadlon and Lamson (2010) who explained the role of sympro constituents especially eucalyptus oil which could react biologically as an antioxidant. The free radicals scavenging capability of *E.tereticornis* oil from fresh and decaying leaves and separate oil constituents potentially leading to oxidative stress (Chirase et al., 2004) could decrease antioxidant capacity and increase lipid peroxidation in the body, thereby increasing susceptibility to disease (Wu, 2016 and Nordqvist, 2017).

Concerning to cellular immunity of the diseased calves phagocytic index and phagocytic percent showed significant decrease. These results agree with Chirase et al., (2004) and Carroll et al., (2007). Also, Sulpizio et al., (2003) who reported that heifers subjected to natural exposure of BRD is often characterized by immunosuppression caused by environmental stressors coupled with a primary viral infection that allows a secondary bacterial infection to establish in the pulmonary tissues. Treated groups with draxxin and draxxin plus meloxicam

compared with diseased group revealed that cellular immunity (phagocytic index and phagocytic %) recorded significant increase in treated groups with draxxin plus meloxicam. This result similar to Faris *et al.*, (2010) and Eleiwa *et al.*, (2014). Also, treated group with draxxin plus sympro revealed that cellular immunity (phagocytic index and phagocytic%) recorded significant increase when compared with diseased group. Our results agree with Abd El-Motaal *et al.*, (2008) who explained that, plant essential oils have been used as pharmaceuticals in alternative medicine and as natural therapy and symopro is a natural product based on the essential oils (Eucalyptus, Lemon oil, Cornment oil, Rosemary and Echinacea purpurea) which created especially for dealing with all respiratory symptoms and has a unique expectorant action and has a powerful immune stimulant effect.

It could be concluded that, no adverse reactions related to sympro were reported during the experimental period. Also, use of draxxin plus sympro had a great effect on reduce the respiratory manifestation and correction of blood gases.

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