academicJournals

Vol. 12(14), pp. 176-182, 15 April, 2018
DOI: 10.5897/AJPP2018.4909
Article Number: 6EF8C4756806
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

In vivo assessment of the antipyretic activity of tilmicosin

A. M. El-Mahmoudy[1], I. M. Gheith[2,3*], A. A. Elmajdoub[4] and S. K. Awidat[4]

[1]Department of Pharmacology, Benha University, 13736 Moshtohor, Egypt.
[2]Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, 344 Medinah, Kingdom of Saudi Arabia.
[3]Department of Biotechnology, Animal Health Research Institute, 11843 Dokki, Egypt.
[4]Department of Pharmacology, Toxicology and Forensic Medicine, University of Tripoli, 13662 Tripoli, Libya.

Received 26 February, 2018; Accepted 10 April, 2018

The aim of the present study was to assess the antipyretic activity of the macrolide antibiotic, tilmicosin, at dose levels of 20 and 40 mg/kg of body weight, subcutaneously, in Brewer's yeast-induced fever model in mice. Pyrexia was induced by subcutaneous injection of 20 mL/kg of 20% (w/v) Brewer's yeast suspension into the animal's scruff region. Eighteen hours later, feverish animals were treated with either tilmicosin or acetylsalicylic acid (200 mg/kg injectable solution, subcutaneously) or vehicle; and rectal temperatures were evaluated at 1, 2, 3, 4 and 5 h post-treatment using digital thermometers. Tilmicosin showed dose-dependent significant decrease in the elevated body temperature of mice that remained sustained throughout the tested time points from 1 to 5 h in the used model. Both small and large dose levels showed a significant inhibition of elevated body temperature when compared with the corresponding febrile controls (37.65 ± 0.04 vs. 38.41 ± 0.08°C and 37.44 ± 0.04 vs. 38.44 ± 0.04, after 1 h; 37.19 ± 0.04 *vs.* 38.41 ± 0.08°C and 36.80 ± 0.03 *vs.* 38.44 ± 0.04°C after 5 h, respectively). These activities were standardized as 38.0 and 51.59% and 47.9 and 66.43% after 5 h, respectively, compared to that of the standard antipyretic and acetylsalicylic acid (200 mg/kg of body weight, subcutaneously). These results may indicate that tilmicosin, in addition to its well established antibacterial activity, possesses significant antipyretic activity that may be beneficial in symptomatic relief when it is used in therapy of infectious disease conditions and inflammatory disorders.

Key words: Antipyretic, tilmicosin, macrolides.

INTRODUCTION

Fever is a well-known finding in almost all of infectious disease conditions and inflammatory disorders. Although uncomfortable, or even risky if 4 degrees over normal because of dehydration, strained heart and impaired respiration; however, it gives an alarm of warning of infection or a risk to the body. Mastering of fever besides treating the specific infectious agent that causes the disease is considered as a critical and important issue in therapy for safety and welfare of animal and human patient subjects.

*Corresponding author. E-mail: e.m.gheith@hotmail.com. igheith@taibahu.edu.sa. Tel: 0096 (014) 861 8888 Fax: 00966 (014) 846 1172.

Classically, the genesis of fever or pyrexia; upon exposure to infectious agents as bacteria, viruses, fungi and some parasites or to mechanical injuries; is induced by inflammatory mediators (that is, prostaglandins and pro-inflammatory cytokines) that are released by affected tissue and activated immune cells (Ahrens, 1996; Roth, 2006). Within the brain, prostaglandin E2 (PGE2), produced by cyclooxygenase (COX)-2, is regarded as the principal mediator of fever (Aronoff and Neilson, 2001) acting on thermosensitive or thermointegrative hypothalamic neurons. Brewer's yeast-induced pyrexia is the most common model for investigating the antipyretic potentials of unknown substances. It is an eukaryote and belongs to the fungus *Saccharomyces cerevisiae* that is single cellular spherical or ellipsoidal in shape. The yeast-induced fever (pathogenic fever) in mouse model was employed in the present study, to investigate the antipyretic activity of tilmicosin.

An effective antipyretic is a drug or agent that can reduce fever or elevated body temperature. The word comes from the Ancient Greek (*anti*, against) and (*pyreticus*, pertaining to fever) (Merriam-Webster, 2014). Antipyretics may interrupt pyrexogenesis at any step from periphery to heat regulating centres. Many well-known and approved antipyretics are established including aspirin, paracetamol, ibuprofen, diclofenac, etc., are prescribed together with specific drugs in fevers associated with inflammatory conditions caused by infectious and/or traumatic agents.

In addition to the standard and established common antipyretic agents, some other drugs may have antipyretic potentials besides their categorized main pharmacological actions and purposes. This finding may give the benefit of synergism when these potentially active drugs can be combined together with the standard ones; synergism may render, sometimes, therapy safer and more agreeable.

Tilmicosin is a macrolide antibiotic with the chemical name of 20-deoxo-20-(3,5-dimethyl piperidin-1-yl) desmycosin. Macrolide class of antibiotics contain a macrocyclic lactone ring in their molecular structure; tilmicosin contains a 16-member one. The kinetic behaviour and properties of macrolide antimicrobials, that is, characterized by a distribution volume (V_d) allow to reach a high concentration in the target tissue (Biophase) even after administration of a small dose. Tilmicosin, among macrolide antimicrobials, is a bacteriostatic and works by penetrating the cell membrane of sensitive microbes and binding to the 50s ribosomal subunit, suppressing protein synthesis. More precisely, the translocation of immature peptide chains between the 50 and 30s ribosomal subunits is interfered, this leads to early detachment and incomplete peptide chains are being synthesized (Seiple et al., 2016). Tilmicosin is an effective remedy for various Gram-positive organisms such as *Corynebacterium* and *Listeria* species, some Gram-negative bacteria, such as *Pasteurella* and *Haemophilus* species, as well as atypical bacteria as *Mycoplasma* species that infection by all of which is associated with fever. Tilmicosin is related to tylosin in its chemical structure, with the chemical formula of $C_{46}H_{80}N_2O_{13}$ and molecular weight of 869.15. From the physical point of view, it is freely soluble in organic solvents like hexane, acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, methanol, and tetrahydrofuran with solubility rate of 1500 mg/L or greater. Water solubility depends on temperature and pH; the solubility rate is about 566 mg/mL at pH 7 and 25°C.

In a previous study, the antinociceptive potential of tilmicosin in mice have been proven against chemical but not thermal stimulai (El-Mahmoudy and Gheith, 2016). The objective targeted in the current study was the assessment of the antipyretic activity of tilmicosin on Brewer's yeast induced fever in mice.

MATERIALS AND METHODS

Tilmicosin

Tilmicosin used in the present study was obtained as the patent drug preparation Pneumotac® (ADWIA, 10th of Ramadan City, Egypt), formulated as 100 mL amber glass vials containing 333.828 mg tilmicosin phosphate/mL, equivalent to 300 mg tilmicosin/mL. To be suitable for mice, the drug solution was diluted in injection sterile water to adjust a mouse dose volume as 0.3 mL diluted solutions that were equivalent to 20 and 40 mg/kg of body weight of mice (small and large dose, respectively).

Acetylsalicylic acid

It was obtained as the patent drug preparation (Aspegic® 500 mg powder for injection, Amriya® Pharmaceutical Industries, Egypt). It was used as a standard antipyretic agent by subcutaneous injection in the scruff region.

Experimental animals

A total of forty male albino mice weighing 25 to 30 g were used for the present study. All animals were maintained on standard pellet diet and water *ad libitum*. Animals were kept under closed environmental conditions of temperature (25°C), humidity (60%) and light/dark cycles (12/12 h). All procedures and experimental protocols are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize suffering of the used of experimental animals.

Experimental design

A parallel design followed in this experiment is as shown in Figure 1. The 40 mice were randomly divided into 5 groups (n = 8 for each) and labelled appropriately. The first group of mice received sterile water prepared for injection (10 mL/kg of body weight), subcutaneously. The remaining 4 groups were rendered feverish by single subcutaneous injection of 20% suspension of Brewer's yeast (20 mL/kg of body weight; Sigma-Aldrich, St. Louis, USA). Animals, then, received different treatments as follows: (i) the first group re-

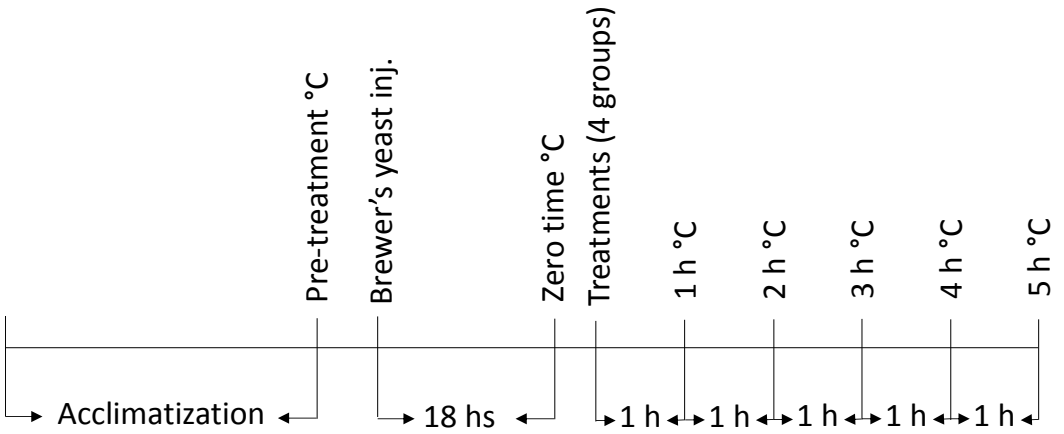

Figure 1. A parallel design is followed in this experiment.

received 0.3 mL injectable sterile water subcutaneously, and kept as control; (ii) the second group received a single small dose of tilmicosin (20 mg/kg of body weight, subcutaneously), adjusted to volume of 0.3 mL with the sterile water used for the control mice; (iii) the third group received a single large dose of tilmicosin (40 mg/kg of body weight) in the same manner; while the fourth group received acetylsalicylic acid (ASA, 200 mg/kg of body weight, subcutaneously in the scruff region as a standard antipyretic drug (Hunskaar and Hole, 1987). The results of the first group were recorded as negative control results to which both test and standard drugs were compared to.

Brewer's yeast-induced pyrexia model

The method described by Tomazetti et al. (2005) was adopted with minor modifications to test the antipyretic activity of tilmicosin in mice. After recording the rectal temperature of each of the animal using a small sized digital thermometer digital thermometer (9SK-1250 MC, Sato Keiryoki Mfg Co. Ltd., Japan), pyrexia was induced by subcutaneous injection of 20 mL/kg of 20% (w/v) Brewer's yeast (Sigma-Aldrich, St. Louis, USA) suspension into the animal's scruff region. Animals were then fasted and 18 h later, the rectal temperature of each animal was re-measured and recorded. Only mice showing an increase in temperature of at least 1°C were used for experiment. Feverish animals were treated with either tilmicosin or standard or vehicle as described earlier; and rectal temperatures were measured at 1, 2, 3, 4 and 5 h post-treatment. Temperature readings of the second group at these different time points were compared to those of the first group; while the readings of the third, fourth and fifth groups were compared to those of the second group at the same time points. Percentage reduction in rectal temperature (antipyresis) was calculated using the following equation:

$$\% \text{ Reduction} = (B - T_n / B - C_n) \times 100$$

where n is the time point = 1, 2, 3, 4 and 5; B is the Brewer's feverish temperature; T is the treated temperature; C is the control temperature.

Data presentation and statistical analysis

All data are expressed as Mean ± standard error of mean (SEM) of eight observations ($n = 8$). Comparison among control and treated groups (small and large doses) were analysed for significance using a one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test as a post-hoc. P-values of 0.05 or less were considered significant. The antipyretic potential of tilmicosin has been, in addition, standardized as % comparable to that of acetylsalicylic acid (standard antipyretic agent). All calculations and procedures of data statistical analysis were carried out using the computer programme SPSS v20 software.

RESULTS

Animals received tilmicosin 20 or 40 mg/kg of body weight exhibited localized tender swellings at the site of subcutaneous injection of both tested doses. Results of the adopted antipyretic test were recorded as the following.

The results of the antipyretic assay of the test (tilmicosin, 20 and 40 mg/kg, SC) and standard (ASA, 200 mg/kg, SC) drugs, as well as those of the control are shown in Table 1 and depicted in Figure 2. Normal rectal temperatures of mice ranged between 36.43 and 36.55°C. All Brewer's yeast suspension-injected animals became feverish after 18 h with rectal temperature range of 38.2 to 38.44°C. Vehicle-treated animals remained feverish until the end of the experimental period. On the other hand, ASA (200 mg/kg of body weight, subcutaneously) as well as tilmicosin (20 and 40 mg/kg of body weight, subcutaneously) have shown effective significant reduction of the elevated rectal body temperatures at all of the tested time points (1 to 5 h) post-administration as compared to the feverish control ($P < 0.05$; ANOVA followed by LSD test). Antipyresis percentage of tilmicosin and those of the standard ASA are shown in Table 2.

DISCUSSION

The chemotherapeutic group of macrolides has been

Table 1. Effects of tilmicosin (20 and 40 mg/kg, SC) and acetylsalicylic acid (ASA; 200 mg/kg, SC) on the feverish temperatures (˚C) induced by Brewer's yeast (20 mL/kg body weight of 20% suspension, SC).

Group	Before yeast	Antipyretic response after Brewer's yeast					
		0 h	1 h	2 h	3 h	4 h	5 h
Control	36.43±0.03	38.20±0.12[a]	38.33±0.12[b]	38.43±0.11[b]	38.45±0.10[b]	38.39±0.10[b]	38.13±0.08[b]
ASA	36.58±0.04	38.54±0.05[a]	36.53±0.03[b]	36.36±0.03[b]	36.28±0.03[b]	36.06±0.04[b]	35.98±0.03[b]
TSD	36.46±0.04	38.41±0.08[a]	37.65±0.04[b]	37.48±0.05[b]	37.34±0.05[b]	37.25±0.04[b]	37.19±0.04[b]
TLD	36.55±0.03	38.44±0.04[a]	37.44±0.04[b]	37.09±0.03[b]	36.98±0.03[b]	36.88±0.03[b]	36.80±0.03[b]

[a]Significantly different from Control; [b]Significantly different from 0 h ($P < 0.05$; ANOVA followed by LSD test); ASA, Acetylsalicylic acid; TSD, tilmicosin small dose; TLD, tilmicosin large dose.

Table 2. Inhibition % of tilmicosin (20 and 40 mg/kg, SC) and acetylsalicylic acid (ASA; 200 mg/kg, SC) on the feverish temperatures induced by Brewer's yeast (20 mL/kg body weight of 20% suspension, SC).

Group	Inhibition % of antipyretic response after Brewer's yeast					
	0 h	1 h	2 h	3 h	4 h	5 h
Control	0.00	-7.34	-12.99	-14.12	-10.73	-1.69
ASA	0.00	102.55*	114.8*	123.47*	126.53*	130.61*
TSD	0.00	38.97*	47.69*	54.87*	59.49*	62.56*
TLD	0.00	52.91*	71.43*	77.25*	82.54*	86.77*

*Significantly different from (0 h of treatment, 18 h after injection of Brewer's yeast) ($P < 0.05$; ANOVA followed by LSD test); ASA, Acetylsalicylic acid; TSD, Tilmicosin small dose; TLD, Tilmicosin large dose.

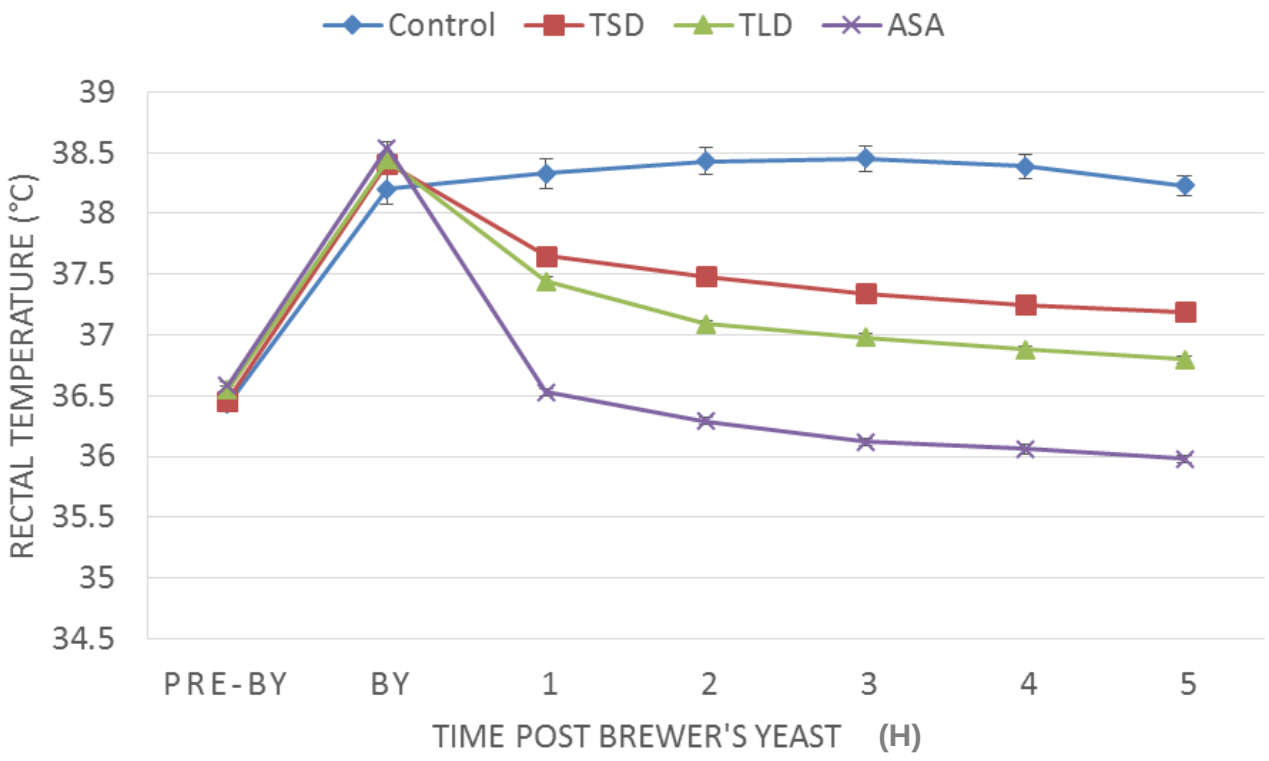

Figure 2. Histogram illustrating the effects of tilmicosin (20 and 40 mg/kg, SC) and acetylsalicylic acid (ASA; 200 mg/kg, SC) on the feverish temperatures induced by Brewer's yeast (20 mL/kg body weight of 20% suspension, SC).

long-used class of antimicrobials and still play an important role in treatment of many diseases caused by sensitive microbes. As they are effective in infective diseases caused by pathogens, intracellularly-penetrating in particular, encouraged the drug specialists for the development of newer derivatives with improved kinetics, dynamics and tolerance (Seiple et al., 2016). However, the penetrating power and the ability of this drug group to accumulate intracellularly (Labro, 1993) may, in addition, cause some alterations in the host cell functions with new interests in their pharmacologic and therapeutic potentials other than antimicrobial one (Bryskier et al., 2008).

The macrolide antibiotic tilmicosin is a tylosin-derivative being used in treatment of respiratory diseases in different animal species. Despite the inflammatory modulating potential of macrolide members, particularly that of erythromycin, has been reported (Labro, 1998; Steel et al., 2012), however, there is no, at least for our information, any data reported about antipyretic potential of this drug group.

Normal body temperature is circadian and varies within about 0.5°C below and above from the morning to the late afternoon (Faull et al., 2015; Hunskaar and Hole, 1987). Thermoregulation is managed by integrated network of neural connections within CNS orchestrated in an area in the hypothalamus named the “preoptic area” which includes the preoptic nuclei located in the anterior hypothalamus (POAH). Simply, the POAH keeps mean body temperature around a particular set-point that is a characteristic for every species. Such normal set-point temperature could be modulated by the balance between functional activities of temperature-sensitive neurons. Such neurons integrate degrees of temperature according to afferent signals from inside the body and its periphery (skin) and evoke various behavioural and physiologic responsive activities integrating heat gain or heat loss (Mackowiak, 1997; Vriens et al., 2014).

Fever or pyrexia is an abnormal rise in the body temperature after an induced increase in the thermos-neutral set-point (Mateusen et al., 2001) by various causes as infections, tissue damages and inflammatory disorders. Upon setting temperature to a higher degree in the hypothalamus, the actual body temperature becomes lower that was set centrally. Consequently, and under the control of the hypothalamus, body physiological and behavioural functional activities favouring heat gain and retention are stimulated until arriving the body at the newly induced raised set-point temperature. Behavioural alterations including shelter seeking and grouping (animals) or adding clothes (human) and physiological alterations, including goose skin, peripheral vasoconstriction, shivering, and non-shivering thermogenesis *via* enhanced release of thyroid, glucocorticoid and catecholamine hormones occur till achieving the raised set-point of body temperature. Now, a condition of fever or pyrexia becomes settled as the thermoregulation mechanism modulates at this higher set point.

Pathophysiological mediators (and hence the pharmacological targets) underlying pyrexia have been described. Endogenous pyrogens including pyrogenic cytokines, like interleukin-1β, tumour necrosis factor (TNF), and interleukin-6, are among those that are acting directly on the hypothalamus to modulate a feverish response (Croft et al., 2000). Exogenous pyrogens, such as surface components of microbes as lipopolysaccharides lipopolysaccharides (LPS), induce fever most probably *via* induction of pyrogenic cytokines capable of functioning at the level of the hypothalamus, in the same way as interleukin-1β (Ramadan, 1997). These signals trigger the release of other mediators, most notably PGE2, in the region of the POAH (Mateusen et al., 2001). Among prostaglandins, PGE2 has been proved to be the downstream mediator of the feverish response. Pre-optic neurons have, on their membranes, E-prostanoid receptors that alter their intrinsic firing rate upon its activation by PGE2, with a result of an elevated febrile set point. Four cellular receptors for PGE2: EP1 through EP4 were proved and reported (McKay et al., 1996; Mora et al., 2013). However, the specific receptor subtype involved in pyrogenesis is still not yet understood.

PGE2 is produced from its precursor arachidonic acid, which is freed from the injured phospholipid cell membranes by phospholipase-A2 (PLA2). Arachidonic acid is a substrate metabolized by COX enzyme that has two isoforms, COX-1 and COX-2. The isoform COX-1 is, usually, expressed constitutively and produces prostanoids important for some housekeeping functions supporting homeostasis as gastric mucosal integrity and renal artery blood flow (Simon, 1999). While the second isoform, COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, interleukin-1β, tumour necrosis factor, and interleukin-6, and bacterial lipopolysaccharide (Simon, 1999). The most likely cell type in the central nervous system responsible for producing PGE2 is the microvascular endothelial cell, which expresses COX-2 abundantly after stress (Cao et al., 1996).

Fever is regulated by the body immune response. Therefore, on the other hand, release of endogenous antipyretic substances is provoked (Jordan et al., 1999) like arginine anti-diuretic hormone (vasopressin), melanocyte stimulating hormone (MSH), interleukin-10 and glucocorticoids act both centrally and peripherally to limit pyrexia. Besides, a group of lipid substances known as epoxy-eicosanoids produced by certain cytochrome P-450 enzymes play an important role in limiting the fever and inflammation (Mackowiak, 2000). Moreover, fever itself appears to counter the release of pyrogenic cytokines (Mackowiak, 1997).

An effective antipyretic may suppress peripheral inflammation and/or central pyrogenic signals. Suppressing central production of PGE2 is a well-

established mechanism of antipyretic drugs, however, stimulated leukocytic and endothelial cells in peripheral inflammatory areas also constitute potential peripheral drug target(s). Two hypotheses may explain the mechanism of antipyretics; the cyclooxygenase-dependent hypothesis and the cyclooxygenase-nondependent hypothesis. In the former, the action of an antipyretic is mediated by inhibiting the production of prostaglandins *via* inhibiting COX as aspirin (Xu et al., 1999). Inhibition of COX-2 by aspirin as well as other NSAID occur via inhibiting its transcription by interrupting a transcriptional activator, named, nuclear factor-kB (NF-kB) (Xu et al., 1999). NF-kB (normally kept inactive in the cytoplasm another protein named IkB) is a heterodimeric protein capable of binding DNA of many genes that are involved in the signalling pathway of inflammatory response. Once involved, NF-kB promotes the transcription of genes encoding pyrogenic cytokines (Kirtikara et al., 2000). The non-cyclooxygenase hypothesis indicates that actions of antipyretics may also be mediated by suppressing tissue inflammation by diminishing leukocyte-endothelial cell interactions (Pierce et al., 1996), reducing pyrogenic cytokine production (Ahrens, 1996), enhancing expression of anti-inflammatory molecules (Ahrens, 1996) or boosting the activity of endogenous antipyretic messengers (Wilkinson and Kasting, 1990).

In the Brewer's yeast-induced pyrexia model, it was found that rectal temperature as well as the percentage of its reduction exhibited by tilmicosin at dose levels of 20 and 40 mg/kg body weight after yeast injection was quite noticeable and comparable to the standard ASA group. The observed antipyretic effect was found to be sustained and lasted up to at least 5 h after single subcutaneous administrations of the drug samples which showed dose-dependent pattern of antipyretic efficacy.

The antipyretic effect of tilmicosin might be due to interruption of pyrexogenesis at any step that connects peripheral inflammation with the central production of PGE2. Although there is no data that supports this speculation, yet the anti-inflammatory effect of macrolides has been reviewed by Labro (1998) who reported that the macrolide antimicrobial drugs have immuno-modulatory potentials; where *in-vitro* data suggested that derivatives of erythromycin may have a direct effect on neutrophil function with altered generation of cytokines involved in the inflammation pathway. In addition, *ex-vivo* data indicated that short-term administration of macrolide(s) might increase the immune response while its long-term administration, in contrast, might result in immuno-suppression. Further studies are needed to improve the current concepts of the pharmacology and therapeutics of macrolides.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahrens FA (1996). Pharmacology. 1st, Wiley, Iowa ed: Wiley. P 328.

Aronoff DM, Neilson EG (2001). Antipyretics: mechanisms of action and clinical use in fever suppression. Am. J. Med. 111:304-315.

Bryskier A, Agouridas C, Chantot JF (2008). New medical targets for macrolides. Expert Opin. Invest. Drugs 3:405-410.

Cao C, Matsumura K, Yamagata K, Watanabe Y (1996). Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1β: a possible site of prostaglandin synthesis responsible for fever. Brain Res. 733:263-272.

Croft A, Duffield T, Menzies P, Leslie K, Bagg R, Dick P (2000). The effect of tilmicosin administered to ewes prior to lambing on incidence of clinical mastitis and subsequent lamb performance. Can. Vet. J. 41:306.

El-Mahmoudy A, Gheith I (2016). The anti-nociceptive potential of tilmicosin against chemical-induced but not thermal-induced pain in mice. Int. J. Immunopathol. Pharmacol. 29:9-16.

Faull O, Cotter J, Lucas S (2015). Cerebrovascular responses during rowing: Do circadian rhythms explain morning and afternoon performance differences? Scand. J. Med. Sci. Sports 25:467-475.

Hunskaar S, Hole K (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30:103-114.

Jordan F, Forrester C, Hodge A, Reeve-Johnson L (1999). The comparison of an aqueous preparation of tilmicosin with tylosin in the treatment of Mycoplasma gallisepticum infection of turkey poults. Avian Disease. pp. 521-525.

Kirtikara K, Raghow R, Laulederkind SJ, Goorha S, Kanekura T, Ballou LR (2000). Transcriptional regulation of cyclooxygenase-2 in the human microvascular endothelial cell line, HMEC-1: control by the combinatorial actions of AP2, NF-IL-6 and CRE elements. Mol. Cell. Biochem. 203:41-51.

Labro M (1993). Intraphagocytic penetration of macrolide antibiotics. Macrolides: chemistry, pharmacology and clinical use. Arnette-Blackwell, Paris, France pp. 379-388.

Labro MT (1998). Anti-inflammatory activity of macrolides: a new therapeutic potential? J. Antimicrob. Chemother. 41:37-46.

Mackowiak PA (1997). Fever: basic mechanisms and management. 2nd ed: Lippincott-Raven Publishers. P 506.

Mackowiak PA (2000). Temperature regulation and the pathogenesis of fever. Princip. Pract. Infect. Dis. 6:703-718.

Mateusen B, Maes D, Hoflack G, Verdonck M, De Kruif A (2001). A comparative study of the preventive use of tilmicosin phosphate (Pulmotil premix®) and Mycoplasma hyopneumoniae vaccination in a pig herd with chronic respiratory disease. J. Vet. Med. B. 48:733-741.

McKay S, Morck D, Merrill J, Olson M, Chan S, Pap K (1996). Use of tilmicosin for treatment of pasteurellosis in rabbits. Am. J. Vet. Res. 57:1180-1184.

Merriam-Webster (2014). Definition of antipyretic [12-2014]. Available from: http://www.merriam-webster.com/dictionary/antipyretic.

Mora N, Uribe-Querol E, Rosales C (2013). Molecular Aspects of Inflammation. In: Pérez-Martínez L, Pedraza-Alva G, Osorio EF, editors. Research signpost. Kerala, India: Research Signpost. pp.15-41.

Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T (1996). Salicylates inhibit I kappa B-alpha phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. J. Immunol. 156:3961-3969.

Ramadan A (1997). Pharmacokinetics of tilmicosin in serum and milk of goats. Res. Vet. Sci. 62:48-50.

Roth J (2006). Endogenous antipyretics. Clin. Chim. Acta 371:13-24.

Seiple IB, Zhang Z, Jakubec P, Langlois-Mercier A, Wright PM, Hog DT, Yabu K, Allu SR, Fukuzaki T, Carlsen PN (2016). A platform for the discovery of new macrolide antibiotics. Nature 533:338.

Simon LS (1999). Role and regulation of cyclooxygenase-2 during inflammation. Am. J. Med. 106:37S-42S.

Steel HC, Theron AJ, Cockeran R, Anderson R, Feldman C (2012). Pathogen-and host-directed anti-inflammatory activities of macrolide antibiotics. Mediators Inflamm. 2012:584262.

Tomazetti J, Ávila DS, Ferreira APO, Martins JS, Souza FR, Royer C, Rubin MA, Oliveira MR, Bonacorso HG, Martins MAP (2005). Baker yeast-induced fever in young rats: Characterization and validation of

an animal model for antipyretics screening. J. Neurosci. Methods 147:29-35.
Vriens J, Nilius B, Voets T (2014). Peripheral thermosensation in mammals. Nat. Rev. Neurosci. 15:573.
Wilkinson MF, Kasting NW (1990). Central vasopressin V1-blockade prevents salicylate but not acetaminophen antipyresis. J. Appl. Physiol. 68:1793-1798.
Xu XM, Sansores-Garcia L, Chen XM, Matijevic-Aleksic N, Du M, Wu KK (1999). Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. Proc. Natl. Acad. Sci. 96:5292-5297.