T.R.
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INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

COMPARISON OF THE EFFECTS OF FLUNIXIN MEGLUMINE AND MELOXICAM ON THE SMOOTH MUSCLES OF THE DIGESTIVE SYSTEM OF MALE CATTLE

Ph.D. Thesis

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ÖZET

FLUNİKSİN MEGLUMİN VE MELOKSİKAMIN ERKEK SIĞIRLARIN SİNDİRİM SİSTEMİ DÜZ KASLARI ÜZERİNE ETKİLERİNİN KARŞILAŞTIRILMASI

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Fluniksin meglumin ve meloksikam güçlü NSAİİ'lerdir. Bu ilaçlar, siklooksijenaz enzimlerini (COX-) inhibe ederek ağrı kesici etkinlik gösterir ve düz kas aktivitesini etkilerler. Bu çalışma, fluniksin meglumin ve meloksikamın erkek sığır sindirim sistemi düz kasları üzerindeki etkisini karşılaştırmayı amaçlamıştır. Çalışmada, mezbahada kesilen erkek sığırlardan doku örnekleri (abomasum, ileum, proksimal loop ve sentripetal girus) toplandı. Toplanan doku örnekleri şeritler halinde kesilerek, izole doku banyosu sistemine asıldı. Karbamilkolin ile düz kas peristaltikleri uyarıldı. Daha sonra banyolara kümülatif olarak atropin, fluniksin meglumin ve meloksikam uygulanarak düz kas aktivitesi ölçüldü. Pozitif kontrol ilacı olarak kullanılan atropin, $1x10^{-5}$ M olan en yüksek konsantrasyonda bile abomazum ve sentripetal girus dokusundaki kasılmaları durduramadı. Eğri altındaki alan (EAA) değerlerine göre, fluniksin megluminin meloksikamdan abomasum, ileum, proksimal loop ve sentripetal girus dokularında sırasıyla 8.57, 4.28, 12.44, 3.93 kat daha etkili olduğu belirlendi. Dakikadaki pik atım sayıları (beat per minute, BPM) yönünden, fluniksin megluminin meloksikamdan abomasum, ileum, proksimal loop ve sentripetal girus dokularında sırasıyla 7.22, 3.88, 7.03, 3.35 kat daha etkili olduğu tespit edildi. Ortalama pik yükseklikleri (peak maximum, P_{MAX}) ile ilgili olarak fluniksin meglumin'in meloksikam'dan abomasum, ileum, proksimal loop ve sentripetal girus dokularında sırasıyla 6.13, 4.43, 7.07, 7.02 kez daha etkili olduğu belirlendi. Sonuç olarak fluniksin meloksikamdan daha etkili ($p<0.001$) olmasına rağmen meloksikam COX-2 enzim seçiciliği nedeni ile daha az istenmeyen etkiye sahiptir. Bu nedenle her iki ilaç da piyasada bulunması ve fiyatına göre sancı kesici amaçla kullanılabilir.

Anahtar Sözcükler: Atropin, fluniksin meglumin, meloksikam, sığır, sindirim sistemi

ABSTRACT

COMPARISON OF THE EFFECTS OF FLUNIXIN MEGLUMINE AND MELOXICAM ON THE SMOOTH MUSCLES OF THE DIGESTIVE SYSTEM OF MALE CATTLE

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Flunixin meglumine and meloxicam are potent nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit the cyclooxygenase enzymes. As an analgesic, flunixin meglumine and meloxicam reduce pain and effect smooth muscle activity. This study aimed to compare the effect of flunixin meglumine and meloxicam on the smooth muscles of the gastrointestinal tract of male cattle. Tissue samples (abomasum, ileum, and proximal loop, and centripetal gyri part of the ascending colon) were collected from the routinely slaughtered male cattle. Collected samples were cut into strips and mounted in a isolated tissue bath system. Smooth muscle contractions were evoked with carbamylcholine. Cumulative doses of atropine, flunixin meglumine and meloxicam were administered to the baths to measure the smooth muscle activity. Atropine, which was used as positive control drug did not abolish the contractions in abomasum and centripetal gyri tissue even at high concentartion of $1x10^{-5}$ M. According to area under curve (AUC) values, flunixin meglumine was more effective than meloxicam 8.57, 4.28, 12.44, 3.93 times on abomasum, ileum, proximal loop, centripetal gyri tissues, respectively. As per beat per minute (BPM) values, flunixin meglumine was more effective than meloxicam 7.22, 3.88, 7.03, 3.35 times on abomasum, ileum, proximal loop, centripetal gyri tissues, respectively. According to peak maximum (P_{MAX}) values, flunixin meglumine was more effective than meloxicam 6.13, 4.43, 7.07, 7.02 times on abomasum, ileum, proximal loop, centripetal gyri tissues, respectively. In conclusion, flunixin was more potent than meloxicam ($p<0.001$). However, meloxicam has lower adverse effects than flunixin meglumine because of its selectivity towards COX-2. Both flunixin meglumine and meloxicam can be used as a pain killer depends on the availability and cost of the respective drug.

Keywords: Atropine, cattle, digestive system, flunixin meglumine, meloxicam

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Saima MUSHTAQ

CONTENTS

ABBREVIATIONS

ACh : Actylecholine

CH : Carbachol

CLM : Confidence of limits

COX : Cyclooxygenase

ENS : Enteric nervous system

GIT : Gastro-intestinal tract

GPCRs : G-protein-coupled receptors

IC_{50} : Fifty percent inhibitory concentration

IV : Intravenously

NSAIDs : Non-steroidal anti-inflammatory drugs

PG : Prostaglandin

PSS : Physiological salt solution

SC : Subcutaneously

FIGURE INDEX

TABLE INDEX

1. INTRODUCTION

As a result of pain, the body shows some biological responses, and prior to which, animals respond in the form of flight or fight or freeze to protect themselves. So, the status of pain can be defined as any feeling as an outcome of tissue damage, leading to physiological, neuroendocrine, and behavioral changes by showing the symptoms of stress as in response (Anderson and Muir, 2005). Similarly, abdominal pain in animals results from several gastrointestinal tract (GIT) conditions like stretching of the mesentery, smooth muscle spasm or contraction, irritation of visceral or peritoneum due to exposure to any chemical. In animals, visceral pain associated with colic having signs of kicking the abdomen and stretching out is transmitted in an autonomic nerve through sensory fibers (Fecteau et al., 2018). On the other hand, gastrointestinal hypermotility is also an outcome of abdominal pain (Holdstock et al., 1969). It is essential to control the pain; if the pain persists for a long time, the effect of some catabolic events increases, and the healing is delayed. To control pain, non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used because of their potent anti-inflammatory and analgesic property, ease of availability, parenteral administration, and duration of action. NSAIDs reduce the movement of smooth muscles and, consequently, pain (Valverde, 2014).

According to their chemical structure NSAIDs are mainly classified as salicylates (sodium salicylate, acetylsalicylic acid), indoleacetic acids (etodolac), indolines (indomethacin), thiopheneacetic acids (diclofenac, eltenac) 2-arylpropionic acids (carprofen, ketoprofen, vedaprofen, ibuprofen), anthranilic acids (flunixin, meclofenamic acid, tolfenamic acid), oxicams (meloxicam, piroxicam, tenoxicam), pyrazolones (phenylbutazone, oxyphenbutazone, isopyrin (ramifenazone), dipyrone) (Lees, 2018).

According to their effect NSAIDs are classified as non-selective COX inhibitors (ibuprofen, and naproxen), preferential COX-2 inhibitors (nimesulide, meloxicam, and etodolac), selective COX-2 inhibitors (celecoxib, rofecoxib, valdecoxib) with analgesic antipyretics with poor anti-inflammatory action (Calatayud and Esplugues, 2016; Scarpignato and Blandizzi, 2016).

1.1. Pharmacology of NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their action by inhibiting cyclooxygenase (COX) enzymes COX-1 and COX-2, which results in inhibition of prostaglandin (PG) synthesis (Rius and Claria, 2016). Commonly used NSAIDs inhibit both forms of COX with the only difference of their relative potency towards COX-1 and COX-2 (Rang et al., 2018). COX-2 inhibition serves the therapeutic anti-inflammatory, analgesic, and antipyretic properties, while side effects like damage of gastric mucosa and bleeding are mostly the outcome of COX-1 inhibition by NSAIDs (Rang et al., 2018; Wang et al., 2005). Intracellularly both COX-1 and COX-2 can be found within nuclear and endoplasmic reticulum membranes, but the concentration of COX-2 is more than COX-1 in nuclear membrane (Morita et al., 1995).

As per their pharmacokinetic profile, NSAIDs are lipid-soluble drugs absorbed readily in oral administration with high bioavailability of 80-100%, but absorption rate varies within species, pH, gut motility, feeding, etc. (Lees, 2018). Topical penetration of NSAIDs in inflamed joints and tissues is minimal, while in synovial fluid, their concentration is somehow detectable. The rate of binding to plasma proteins is high, about 95-99%. Biotransformation of mostly NSAIDs is carried out by both phase 1 (oxidation, hydroxylation, demethylation) and phase 2 (glucuronidation, other conjugations) mechanisms. Clearance of the drug from plasma is mainly through hepatic biotransformation accompanied with renal excretion of their metabolites (Calatayud and Esplugues, 2016).

Therapeutically NSAIDs provide anti-inflammatory, antipyretic, and analgesic properties. NSAIDs are potentially used for treating chronic conditions like osteoarthritis, rheumatoid arthritis. They are also used in the treatment of rheumatic fever, gout, acute pain, dysmenorrhea, and also for prophylactic purposes for the treatment of myocardial infarction and stroke (Aksoy and Das, 2021).

NSAIDs inhibit prostaglandin (PG) synthesis, which leads to several adverse effects, e.g., gastrointestinal (GI) toxicity, cardiovascular adverse effects, renal toxicity, fluid retention, and the exacerbation of hypertension. COX-1 inhibition by NSAIDs is mainly responsible for GI toxicity which results in GI ulceration, severe

perforation of the upper GI tract, and bleeding of the gastrointestinal tract (Bacchi et al., 2012)

Cardiovascular side effects associated with NSAIDs therapy include oedema, congestive heart failure, hypertension, stroke, and other thrombotic events (Wongrakpanich et al., 2018), while renal side effects of NSAIDs include renal dysfunction, nephrotic syndrome, fluid and electrolyte disorders, and renal papillary necrosis. Also, NSAIDs can adversely effect blood pressure control, mainly during the use of angiotensin-converting enzyme (ACE) inhibitors, diuretics, and β blockers (Whelton, 1999).

Earlier NSAIDs selectivity was determined by different pharmacological tests based on a comparison of gastrointestinal damage with pain-antagonizing, fever, and inflammation-reducing activities. These studies were found to be more time-consuming and more usage of live animals, hence later replaced by in *vitro* inhibition studies to analyse the potency or inhibitory concentration (IC_{50}) and NSAIDs selectivity towards COX-1 and COX-2 isoforms (Beretta, et al., 2005).

The COX-1 and COX-2 ratio defines the selectivity of NSAIDs. It refers to the dose of the drug to be given to inhibit both isoforms. Non-selective NSAIDs have a COX-1: COX-2 ratio close to 1, while COX-2 inhibitor drugs possess a ratio higher than 1, which means a higher dose of drug must be given to inhibit COX-1 compared to the dose required to inhibit COX-2 (Weir, 2014).

1.2. Muscarinic receptors

Acetylcholine (ACh) is one of the major neurotransmitters of the nervous system. It acts as an agonist on two types of cholinergic receptors, nicotinic (N) and muscarinic (M). N receptors are mainly found on postganglionic neurons in the autonomic ganglia of the autonomic nervous system (ANS), while M receptors are abundantly found in postsynaptic sites like the brain, heart, respiratory system, gastrointestinal system, and bladder. (McMurphy et al., 2019). M receptors mediate various physiological functions depends on the subtype of receptor and location. There are mainly five subtypes of muscarinic receptors found, M1, M2, M3, M4, M5. M receptors present in the brain helps in neuronal excitability and release of ACh. M2 receptors at the heart, on activation, reduces the heart rate. (Abrams, et al.,

2006). In central nervous system (CNS) M5 receptors potentiate the release of dopamine (Shin et al., 2015).

Muscarinic receptors type M2 and M3 are found in smooth muscles of GIT. Contractions of GIT smooth muscles are mainly stimulated by muscarinic receptor agonists (Ehlert et al., 1997). The ratio of M2:M3 receptors in GIT is 4:1 approximately (Niederberger et al., 2010); however, receptor responsible for eliciting the contractile response had pharmacological properties consistent with that of the M3 receptor. Muscarinic receptors distribution differs from species to species on GIT. For example, on ileac tissues of rat, Guinea pig and pig M2 receptors are major while M1, M3 and M4 receptors are minor subtype of muscarinic receptors present (Ehlert et al., 1997). Similarly muscarinic receptor subtypes M1, M3, and M5 were found on smooth muscle cells of the gastrointestinal tract of healthy dairy cows (Stoffel et al., 2006). In most GIT isolated tissue preparations, muscarinic agonist evokes contractions by acting on M3 receptor-like carbachol (CH), a muscarinic receptor agonist drug which induces contractions in smooth muscle by acting on M3 receptors (Kitazawa et al., 2007). Moreover, unlike other cholinergic drugs, CH is resistant to be destroyed by cholinesterases enzyme, and the drug is somehow active on both M and N receptors (McMurphy et al., 2019). Intracellularly M3 receptors stimulate hydrolysis of phosphoinositide (PI) and cause calcium ion (Ca^{2+}) mobilization by interacting with guanosine triphosphate-binding protein (G-proteins) of Gq family while M2 receptors coupled with inhibitory G protein (Gi) family and help in pertussis toxin-sensitive inhibition of cyclic adenosine monophosphate (cAMP) accumulation (Ehlert et al., 1999).

G-protein-coupled receptors (GPCRs) are the largest class of membrane proteins. They regulate various cellular functions via stimulatory/inhibitory G-protein (Gs/i)/cAMP and Gq G-protein/calcium pathways. About 30% of drugs mediate their actions by interacting with GPCRs (Liu, et al., 2010). The physiological effects of PGs are also mediated by G-protein-coupled prostanoid receptors, a family of rhodopsin-like GPCRs. Prostanoid receptors are further categorised into eight subtypes, based on their ligand-binding affinity named as D prostanoid (DP), E prostanoid (EP1, EP2, EP3, EP4), I prostanoid (IP), and thromboxane (TP) (Hata and Breyer, 2004).

1.3. Role of prostaglandins in GIT

Prostaglandins can be found in the GI mucosa of both animals and humans. The occurrence of PGs in the gut differs from species to species; for example, PGE2 is mainly found in human tissue, while animals have both PGE2 and PGF2 (Waller, 1973). PGs play an important role in the physiology and pathophysiology of GIT by effecting motility along with other processes, extensively found throughout the GIT, mainly in smooth muscles of the stomach and small intestine (Grasa et al., 2006). PGs are formed as a result of the release of arachidonic acid (AA) 20-carbon unsaturated fatty acid from the plasma membrane by phospholipase. The main PGs generated are PGE2, PGI2, PGD2, and PGF2α and thromboxane (TxA2) (Ricciotti, et al., 2011). PGE, PGD, PGI, PGF, and TX having specific type receptors defined as EP, DP, FP, and IP receptors (Ruan et al., 2011). PGE2 exerts their action by interacting with GPCRs at the plasma membrane. PGE2/EP acts through its four types of nuclear membrane G protein receptors named EP1, EP2, EP3, and EP4 with distinctive signalling pathways. EP1 receptors coupled with Gαq-protein causes hydrolysis of PI, which eventually increases Ca^{2+} mobilization intracellularly. Activation of EP3 and EP4 receptors after interaction with Gαs (stimulatory G-protein) cause an intracellular increase of cAMP level while EP3 decreases the level of cAMP after activating α-subunit of inhibitory G-protein (Gαi) (De Keijzer et al., 2013). In smooth muscles, PGs induce contractions and relaxations by acting on receptors. Relaxation of muscles occurs as a direct action of PGs on the receptors, while excitation can occur either through intrinsic nerves or at non-neural sites (Waller, 1973). The stimulatory effect of DP, EP2, EP4, and IP receptors results in the relaxation of smooth muscles by increasing cAMP level intracellularly while EP1, FP, and TP receptors are coupled to Ca^{2+} mobilization hence induce smooth muscle contractions (Ruan et al., 2011).

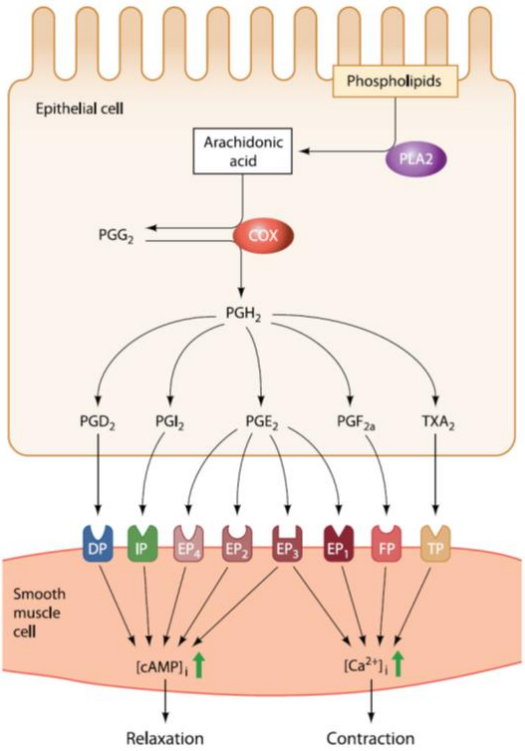

Figure 1.1. Responses to PG production, different PG receptors and their effects on smooth muscles (Ruan, et al., 2011)

Different studies have been reported the release of PGs both in *vitro* and in *vivo* in GIT tissues depends on the type of preparations (type of muscle layer), type of PGs, species used, and ability of the assay to detect them (Waller, 1973). Stated by (Eberhart and Dubois, 1995) in some in *vitro* studies on intestinal tissue, activation of E_2 receptor have a contrasting effect on different layers of smooth muscle. In the longitudinal muscle layer, E2 activation induces contraction, while in circular muscles, it induces relaxation. Also, PGs availability differs from species to species. For example, Tx level is much lower in horses and pigs as compared to dogs and cattle, while PGE2 level is quite similar in all four species (Beretta et al., 2005).

1.4. Aim of study

As a result of pain in the gastrointestinal tract caused by numerous factors, NSAIDs are used. NSAIDs reduce the movement of smooth muscles and consequently pain by suppressing the cyclooxygenase enzyme (COX), enabling the synthesis of prostaglandins from arachidonic acid. Previously in *vitro* studies have been investigated to analyse the effect of NSAIDs on the motility of gut smooth muscles, and different tissues from different animals have been used, for example,

rabbit ileum, guinea pig ileum, duodenum, the stomach of mice etc and every isolated tissue from any specie will give response to the applied drug if kept in suitable physiological salt solution (PSS). Among all the studies reported on isolated tissues using different NSAIDs, there is no reference available showing the comparative effect of two NSAIDs, flunixin meglumine and meloxicam, directly on the gut motility of bovine. This study is prepared to examine the comparative effects of flunixin meglumine and meloxicam on peristaltic movements of the digestive system of male cattle. Moreover, the IC_{50} and efficacy difference of flunixin meglumine and meloxicam is examined in an isolated tissue bath system by using the tissues obtained from four different regions of the digestive system (abomasum, ileum, and proximal loop, and centripetal gyri part of ascending colon) of male cattle. Atropine is used as positive control drug, while carbamylcholine is used as a stimulant drug.

2. GENERAL INFORMATION

2.1. The effect of NSAIDs on gastrointestinal motility

Along with their analgesic property, NSAIDs also effect the activity of gut smooth muscles. Different studies conducted on large and small intestine tissues have shown the inhibitory effect of NSAIDs on intestinal motility (Marshall & Blikslager, 2011). In GIT, prostaglandin inhibition through NSAIDs causes an inhibitory effect on the function of GIT, which results in contractile activity of gut tissue (Hoogmoed, et al., 2000). In *vitro* investigation of NSAIDs, all NSAIDs decrease the contractions of smooth muscles in the colon of horses. In colon samples of dogs and humans, indomethacin decreases the peristaltic movement, while in the small intestine of guinea pig, it induces phasic contractions (Hoogmoed et al., 2004). In *vitro* study on intestinal smooth muscle preparation of rabbit, meloxicam and piroxicam have shown an inhibitory effect on tissues (El-Rwegi, et al., 2015).

Among NSAIDs flunixin meglumine is frequently used in large animals to reduce the pain related to colic. While providing analgesia, the drug also effects the gut smooth muscles activity (Menozzi et al., 2009). In *vitro* preparation of GIT tissues of bovine, a clear contractile effect was observed after applying the flunixin meglumine. However, the immensity of the effect was different between gut and segments (Mendel et al, 2018). Similarly on investigating the effect of NSAIDs on ileac motility of horse indomethacin shows no inhibitory effect while flunixin meglumine inhibits the tonic contractions, and celecoxib also decreases the contractile activity of ileal tissues (Menozzi et al., 2009).

2.2. Isolated tissue bath system

In *vitro* study with isolated organs/tissues was first introduced by Vergl. R. Magnus, nowadays which has been used extensively to study the drug-receptor interactions (de Graaf et al., 1983). Isolated tissue bath is set of apparatus which permits the pharmacologists, physiologists, or biochemists to study an isolated tissues in-*vitro*; under controlled utmost close to the physiological conditions to get useful information of drugs or response of the tissues to certain drugs (Jespersen et al., 2015). This technique is gaining popularity at the international scientific level due to its lower cost, use of few animals, and significant results in a short period of time as compared to in *vivo* techniques (Ramirez et al., 2007).

Figure 2.1. Photo of the isolated tissue bath system

Control of the physiological variables like temperature, pH, oxygen, and including substrate delivery, are some of the major basic requirements of the isolated tissue bath system. Temperature is controlled via a water-jacketed tissue bath, as illustrated in figure 2.2 (Jespersen et al., 2015).

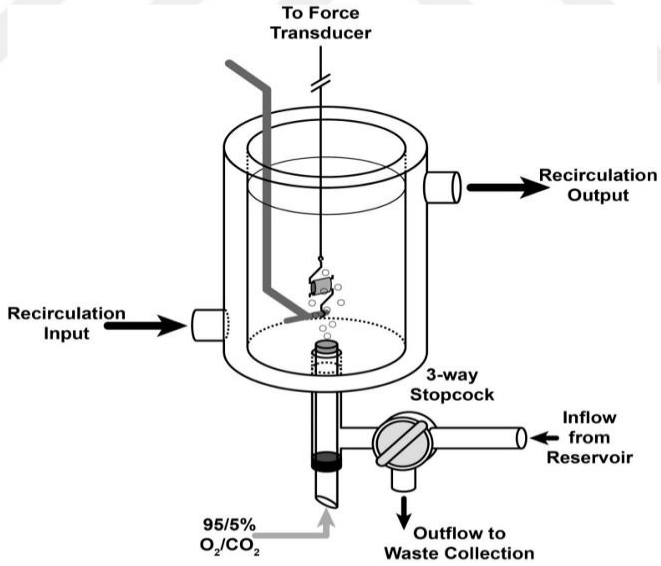

Figure 2.2. A schematic representation of double-walled water jacketed tissue bath. Buffer flow is regulated by a 3-way stopcock and rest of the connections are illustrated well (Jespersen et al., 2015)

In isolated tissue studies, the tissue is isolated from the body and kept in physiological salt solution (PSS), which constitutes of different chemicals as

nutrients that maintain the tissue's viability in a suitable environment and temperature. pH of the PSS is adjusted close to 7.4, which maintains the normal anion homeostasis. During the experiment, PSS is treated with carbogen (95% O_2 and 5% CO_2), mimicking the body's physiological environment (Agnes and Liang, 2007).

Additional requirements may include electrical stimulation of tissue and changes in flow, pressure, or distention, which can be done by controlling various parts of the instrument and recorded by the experimental setup. Transducers/probes of various sizes can be used to record results, depends on the tissue type, size, and type of the experiment being investigated (Robinson, 2013). Mostly there are two types of transducers. (i) isotonic transducer gives the result in case of any change in muscle length during contraction, and (ii) isometric transducer, which records the force developed by the tissue when its length is maintained constant. Results can be analysed and recorded by computer software. (Salmon, 2014).

Figure 2.3. Photo of data acquisition system and transducer amplifier

In *vivo* experiments, gastrointestinal motility is influenced by an external factor like circulating hormones, the effect of extrinsic nerves, and control of reflexes that arises from other regions of the gut. However, isolated tissue experiments somehow overcome these problems but lead to some other factors. For example, perfusion of

the tissue via blood vessels is not exactly the same as in *vivo*, and with a lack of blood supply, the tissue becomes hypoxic. Moreover, in *vitro* muscle contraction is not necessarily match up with the contractive efficiency of in *vivo* experiments. With all these limitations, an isolated bath system is still considered an ideal tool to study drug response in different tissues of an animal model (Pozzoli and Poli, 2010).

2.3. Flunixin meglumine

Flunixin meglumine is 3-Pyridinecarboxylic acid, 2-[[2- methyl-3-(trifluoromethyl)phenyl]amino]-, compounded with 1- deoxy-1-(methylamino)- D-glucitol (1:1) possessing molecular formula $C_{14}H_{11}F_3N_2O_2$ and molecular weight of 491.46 (Malik, 2017). Its usage in cattle is approved by the Food and Drug Administration (FDA) (Stock, 2015). Flunixin meglumine is a potent NSAID that suppresses the COX enzymes in the arachidonic acid pathway, thereby reducing the formation of PGs and creating a pain reliever and antipyretic effect (Hassan et al., 2016). In cattle flunixin meglumine shows an effect by inhibiting selective COX-1 (Lees, 2008). COX-1 is a constitutive enzyme, mediates the release of PGs. These PGs help to regulate the production of cytoprotective gastric mucus. Inhibition of this isoform led to undesirable effects like gastric injury, ulceration, and delayed blood clotting (Whittle, 2000).

In cattle flunixin meglumine is administered at a dose of 2 mg/kg with a plasma half-life of 3-4 hours via intravenous (IV) administration with a 60% oral absorption rate (Papich, 2016).

Flunixin meglumine has shown an anti-endotoxic effect in various experimental septic shock models (Milovanovic et al., 2016). NSAIDs are most effective in somatic and skin-bone pain; their pain relief activities are weak in the internal organs, while flunixin meglumine shows high pain relief activity in colic-related internal organ pain. It is also used to treat inflammation associated with respiratory system infections, endotoxemia or mastitis in cattle (Hsu and Kanthasamy, 2008). In cattle, it is also used in combination with some antibiotics for the treatment of blackleg, vaginal prolapse, and pneumonia (Malik, 2017).

2.4. Meloxicam

Meloxicam, also known as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2-H-1,2-benzothiazine-3-carboxamide 1,1-dioxide, having a molecular formula of

$C_{14}H_{13}N_3O_4S_2$ and a molecular weight of 351.41 (Cooper, et al., 2013). Meloxicam exhibits pain-relieving and anti-inflammatory effects by suppressing the PG synthesis by inhibiting the COX enzyme. The COX-2 enzyme subtype mediates the synthesis of PGs which are responsible for side effects of the inflammatory reaction like fever and pain in the organism (Papich, 2016). Meloxicam inhibits the selective COX-2 enzyme, inhibition of this isoform results in more therapeutic with lesser adverse effects, especially on the gastrointestinal tract. (Ogino et al., 2000).

Meloxicam is used at a dose rate of 0.5 mg/kg subcutaneously (SC) or IV in cattle. Drug shows a prolonged half-life (27 h (range 19.97-43.29 h) and higher bioavailability on oral administered in cattle. Many studies reported usage of meloxicam as an analgesic in cattle due to its significant properties (Stock, 2015: Plumb, 2011).

Meloxicam is also used in acute or chronic skeletal-muscle disorders and to reduce postoperative pain and inflammation after orthopaedic and soft tissue surgery. The application of meloxicam in animals with kidney disease is not recommended before surgery but can be used after careful evaluation (Ramsey, 2008). Meloxicam is a relatively safe NSAID, but its use in pregnant, nursing or animals under four months is prohibited. Digestive system disorders such as vomiting, loss of appetite, and diarrhoea are the most frequently reported undesirable effects, probably due to inhibition of the COX enzyme (Hsu and Kanthasamy, 2008).

Various interactions may occur between meloxicam and other drugs. Some have been reported to occur in humans and animals theoretically. Angiotensin-converting enzymes (ACE), such as enalapril and benazepril, may reduce the blood pressure-lowering effect of suppressing drugs. It can increase the risk of bleeding by strengthening the effect of drugs that increase the fluidity of the blood, such as heparin and warfarin. Aspirin may cause ulcers, bleeding, vomiting, and diarrhoea in the gastrointestinal tract. Interaction with corticosteroid drugs such as prednisolone may increase the severity of unwanted effects in the digestive system (Plumb, 2011).

Table 2.1. Some pharmacokinetic and pharmacodynamics values of flunixin meglumine and meloxicam in cattle

Drug	Dose[2]	$t_{1/2}$ (h)[2]	F(%)[5]	t_{max}(h)[5]	MRLs (µg/kg)[3,4]	Target tissue	IC_{50} COX-1 / IC_{50} COX-2	References
Flunixin meglumine	2.2 mg/kg IV	3.8	60%	3.5± 1.0	20	Muscle[4]	0.606[6]	Beretta et al. 2005[1] Coetzee, 2013[2] EMEA, 1997[3] EMEA, 2000[4] Stock, 2015[5] Miciletta, 2015[6]
					30	Fat[4]		
					300	Kidney[4]		
					100	Liver[4]		
Meloxicam	0.5 IV, SC 0.5–1 mg/kg oral	27	100%	11.6 h	203	Muscle[3]	3.806[1]	
					603	Liver[3]		
					353	Kidney[3]		

2.5. Atropine

Atropine being a parasympatholytic/anticholinergic drug, inhibits the action of ACh on smooth muscle cells. In GIT, atropine causes the relaxation of smooth muscles by blocking the effect of cholinergic nerve impulses, which make it beneficial to treat hypermotility and intestinal spasms. Along with other parts of the stomach and intestine, atropine can also cease rumen motility (McMurphy et al., 2019). Apart from decreasing motility atropine act as an antimuscarinic drug that inhibits the stimulation of cholinergic agent, thus reducing gastrointestinal secretions, inducing heart rate and mydriasis. In case of excess vagal stimulation, atropine acts as a drug of choice, also used in the intoxication of organophosphate poisoning. In ruminants, atropine is administered at a dose of 0.1 mg/kg IV for organophosphates poisoning and 0.02 mg/kg IV or 0.04 mg/kg IM preoperatively as anesthesia adjunct (Papich, 2016).

Atropine activity can be enhanced in interaction with antihistamines, benzodiazepines, procainamide-type drugs, while prokinetic drugs like metoclopramide are antagonised by atropine (Rock, 2007).

2.6. Anatomy, physiology, and histology of the ruminant digestive tract

2.6.1. Anatomy

The stomach of the ruminants found on the left side of the abdominal cavity occupies about 3/4 of the total space (Dehority, 2002). It consists of four parts,

rumen, reticulum, and omasum, generally known as the forestomach, while the fourth part, abomasum is considered as glandular stomach (Clauss and Hofmann, 2014). The rumen is the largest compartment of compound stomach animals, although its size differs from species to species. It is roughly ovoid, compressed on the lateral side, while the internal part is divided into dorsal and ventral sacs by series of shelf-like pillars (Harfoot, 1978). Contraction of the sacs is supported by these pillars, which further helped in the mixing and circulation of ingesta (Dehority, 2002). The reticulum is somehow related to the rumen in its function and structure, in combination described as rumino-reticular compartment by some authors. Spherical reticulum can be seen on cranial to lateral in contact with the caudal surface of the diaphragm. Omasum can be found on the right side of rumino-reticular compartment, which lies within the intrathoracic part of the abdomen (Sautet and Liebich, 2004). The abomasum is a thin J-shaped wall structure located on the floor of the abdominal cavity. It is grossly divided into the fundus, body, and pyloric regions (Dyce, et al., 2009). Fundus lies in the xiphoid region, somehow connected to the reticulum and ventral sac of rumen. The body lies more towards the left side of the median plane and extends caudally, and the pyloric region is dorsally inclined and is connected to the duodenum, part of a small intestine (Weyns, 1988).

The intestines (small and large) can be seen completely to the right of the midline, mainly to the dorsal part of the abdomen and in part lie under the ribs cover (Dyce, et al., 2009). The small intestine, with a total length of 27-49 m, comprising 3 parts duodenum, jejunum, and ileum. The jejunum is the largest part of the small intestine. In ox ileum, part of the small intestine has a 1 cm long straight part near the caecum. Similarly, the large intestine consists of the caecum, colon, and rectum. The length of the colon is about 7-9.5 m which is further comprised of ascending, transverse, and descending colon. Ascending colon is the longest part of the large intestine consists of proximal and spiral loop of colon, centripetal gyri, central flexure, and centrifugal gyri (Simoens et al., 2003).

2.6.2. Physiology

Reduction in the particle size of the ingesta is a basic achievement of the digestive physiology of ruminants which increases intake and makes digestibility easier for them (Clauss and Hummel, 2017). Microbial fermentation is one of the

specialities of the ruminant digestive system, which mainly occurs in the rumen part of the stomach (Niwińska, 2012). Half of all feed digestion (50%) occurs in the rumen. Rumen walls possess finger-like projections known as papillae, which increase the absorption of nutrients by raising surface area and having many microbes that help feed digestion. Reticulum and rumen are considered a single chamber referred to as reticulorumen, which is sometimes also considered an absorption chamber and fermentation vat that carries vat fermentation (Tayab, 2019). The reticulum is mostly involved in rumination processes. The Omasum help to filter large particles back to the reticulorumen, thus allow movement of fluid and fine particles to the abomasum. It also helps in the resorption of water and absorption of some volatile fatty acids, while abomasum aid in the production of acid and enzymes for protein digestion.

The small intestine serves as a place for the maximum amount of digestion. Digestion of proteins, starch, and sugars occurs in the small intestine by pancreatic secretions, while bile help in fat digestion (Hall and Silver, 2009). Absorption of water and electrolytes processed in the large intestine. It also serves as storage for fecal material until it is excreted (Guyton and Hall, 2006).

2.6.3. Histology

Histologically GIT consists of four layers (i) mucosa consist of epithelial cells, lamina propria, and muscularis mucosae (ii) submucosa (iii) two smooth muscle layers (inner circular and outer longitudinal layer) and (iv) serosal layer. (Herdt and Sayegh, 2013). The forestomach (rumen, reticulum, and omasum) of ruminants is non-glandular, having keratinized stratified squamous epithelium while the abomasum is lined by glandular mucosa. Ruminal mucosa possesses tongue-shaped papillae, while epithelium of rumen is keratinized stratified squamous, which aids in absorption, protection, and metabolism (Frappier, 2006). The abomasum is a glandular part lined with simple columnar mucus-secreting epithelium. As abomasum consists of three parts cardia, fundus, and pylorus, glands are sparse with few cells in the cardia but are abundant and cellular in the fundus.

The small intestine possesses finger-like projections of intestinal villi, thick in ruminants, increase the surface area for absorption (Aughey and Frye, 2001). Villis surface is lined by simple columnar epithelium and abundant goblet cells. Smooth

muscles are present in the tunica muscularis layer of the small intestine. On the other hand, the large intestine lacks the villi. The number of goblet cells is increased compared to the small intestine that helps in lubrication by producing more mucus (Eurell, 2004).

2.7. Neuronal control of GIT

Gastrointestinal tract, only system having an independent nervous system called enteric nervous system (ENS), which functions independently without any input from the central nervous system (CNS). Many studies discovered the presence of peristaltic in isolated intestine tissue without any signal from CNS as ENS is capable of generating complex neurogenic motor patterns (Spencer and Hu, 2020).

GIT motility mainly requires neuronal control, chemical control, and myogenic control. Neuronal control includes both intrinsic as well as extrinsic systems. Intrinsic innervation involves ENS, while extrinsic innervation involves splanchnic and vagus nerve supply to the stomach and upper intestine and pelvic nerve supply to a portion of distal intestine. The ENS comprises of ganglia which are located in the myenteric and submucosal plexuses interconnected to nerve strands. The movement of smooth muscles are controlled by extrinsic neurones of the sympathetic and parasympathetic system via neurones of the myenteric plexus (Hansen, 2003; Steiner and Roussel, 1995).

Chemical control of GIT is through several neurotransmitters and neuromodulators released from nerve endings of the autonomic nervous system and smooth muscle cell nerve endings (Steiner and Roussel, 1995). GIT neurotransmitters are both excitatory e.g., serotonin (5-hydroxytryptamine, 5-HT) and acetylcholine, and inhibitory like noradrenaline and nitric oxide (Hansen, 2003). The activity of ENS and motility is evidently influenced by the excitatory neurotransmitter serotonin (5-HT). In isolated intestinal tissues, modulation of 5-HT has induced segmental contractions. However, these contractions are in differ with peristalsis, as segmental contractions are caused by as a result of electrical rhythmicity in the smooth muscle cell having no dependence on nervous function, while peristalsis occurrence is highly dependent on the function of ENS (Spencer and Hu, 2020).

3. MATERIALS AND METHOD

3.1. Chemicals and incubation medium

Carbamyl-β-methylcholine chloride, meloxicam, atropine sulfate (Sigma Chemicals Co, St. Louis, USA), flunixin meglumine (Dr. Ehrenstorfer GmbH,Augsburg, Germany), D-glucose, $MgSO_4$, $CaCl_2$, KCl (Merck, Darmstadt, Germany), Nacl, $KH2PO_4$ (Isolab Laborgeräte GmbH, Germany) were used as experimental drugs to conduct the experiment. For incubation and transportation of tissue samples, PSS with a composition of 116 mM NaCl, 4.6 mM KCl, 1.16 mM NaH_2PO_4 x 2H2O, 1.16 mM $MgSO_4$ x 7H2O, 21.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$ x 2H2O and 11.6 mM dextrose having pH of 7.4 was prepared on a daily basis.

3.2. Gastrointestinal tissue collection and preparation

Tissues were collected from different healthy male cattle that go through routine slaughtering. Abomasum tissues were taken from the pyloric antrum of the abomasum, segments of ileum were collected from 30 cm proximally to the caecum, whereas samples of the colon were collected from the proximal loop and centripetal gyri part of ascending colon. Collected tissues were carried to a laboratory in a cold chain immediately after collecting from the slaughterhouse. At the laboratory, tissues were prepared with measurements of 1 cm x 0.5 cm x 0.2 cm in length, width, and thickness, respectively. Prepared tissue strips were hung vertically and attached to force transducers (Commat, Turkey) with basal tension of 2 g in 10 ml tissue baths (Commat, Turkey) with supply of (95% O_2 + 5% CO_2, pH 7.4, 38°C).

3.3. Measurement of smooth muscle activity

Thirty muscle strips from four different parts of GIT mentioned above were tested for each drug (flunixin meglumine, meloxicam, and atropine) in total; 120 muscle strips were used taken from different 30 male cattle. All samples were incubated for 2-4 hours, and the bath solution was changed after every 15 minutes during this incubation period. Four muscle strips were mounted in four different baths simultaneously. After system stabilization, the mechanical activity was measured using an isotonic transducer connected to the preparation, developing a passive stretch throughout the experiment. Tissue viability was confirmed by observing the occurrence of measurable spontaneous contractions with a data acquisition system (Biopac, USA) for the period of 20 minutes. After that, a

stimulatory drug (CH) at an effective concentration of $1x10^{-5}$ M was applied to all four baths to evoke the contractions. This concentration of CH was determined based on the results of preliminary studies. After receiving maximum contractions from every tissue in response to CH, an experimental drug was applied. Each drug was analysed individually on every tissue. Concentrations of atropine applied to each muscle bath were $1x10^{-11}$, 1x10-^{10}, $1x10^{-9}$, $2x10^{-9}$, $3x10^{-9}$, $4x10^{-9}$, $5x10^{-9}$, $6x10^{-9}$, $7x10^{-9}$, $8x10^{-9}$, $1x10^{-8}$, $1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ M. Flunixin meglumine were applied at concentrations of $1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$, $2x10^{-5}$, $3x10^{-5}$, $4x10^{-5}$, $5x10^{-5}$, $6x10^{-5}$, $7x10^{-5}$, $8x10^{-5}$, $9x10^{-5}$, $10x10^{-5}$ M while concentrations of meloxicam applied to each muscle bath were $1x10^{-6}$, $1x10^{-5}$, $1x10^{-4}$, $2x10^{-4}$, $3x10^{-4}$, $4x10^{-4}$, $5x10^{-4}$, $6x10^{-4}$, $7x10^{-4}$, $8x10^{-4}$, $9x10^{-4}$ M. After each drug application contractions were recorded for period of 20 minutes.

3.4. Data analysis

Mean and standard error of mean (SEM) values of area under the curve (AUC), frequency/beat per minute (BPM), and peak amplitude (P_{MAX}) were calculated for 120 GIT tissue samples obtained from 30 male cattle. Inhibitory concentration (IC_{50}) of atropine, flunixin meglumine and meloxicam and comparison between the mean IC_{50} values of AUC, BPM and P_{MAX} for tissues and drugs were calculated by using non-linear regression of GraphPad Prism 9 software. Analysis results were expressed as means (95% confidence of limits, CLM). Differences were considered as statically significant in case of P value was less than 0.05 ($p<0.05$) and F is considered as analysis of variance test statistics.

4. RESULTS AND DISCUSSION

The present study is designed to investigate the comparative effect of flunixin meglumine and meloxicam on the smooth muscles of the digestive system of male cattle while atropine is used as positive control drug. Collected tissues were analysed in an isolated tissue bath system.

4.1. Results

Atropine at a higher concentration of $1x10^{-5}$ M did not abolish the CH evoked contractions in the pyloric antrum of the abomasum and centripetal gyri of ascending colon as shown in figure 4.1 and 4.4, while contractions in the ileum and proximal loop of ascending colon were blocked by atropine at $1x10^{-9}$ M shown in figure 4.2 and 4.3. The parameters calculated to infer the drug result were IC_{50} values of area under curve (AUC), frequency/beat per minute (BPM), and peak amplitude (P_{MAX}) presented in Table 4.1.

The second experimental drug flunixin meglumine, abolished the contractility of all four bovine GIT preparations. In abomasum, ileum, proximal loop, and centripetal gyri specimens, flunixin meglumine showed a clear inhibitory effect at concentration $6x10^{-5}$ M shown in figure 4.5, 4.6, 4.7, and 4.8. The parameters calculated to conclude the drug result are IC_{50} values of AUC, BPM, and P_{MAX} presented in table 4.1.

Similarly, meloxicam inhibit the CH evoked contractions in all GIT preparations. In abomasum, ileum, proximal loop, and centripetal gyri preparations, an inhibitory effect was noticed if meloxicam was administered at concentration of $4x10^{-4}$ shown in figure 4.9, 4.10, 4.11, and 4.12. IC_{50} values of AUC, BPM, and P_{MAX} for meloxicam are presented in table 4.1.

Differences in the IC_{50} values of meloxicam/flunixin meglumine for all three parameters were calculated, presented in Table 4.2. According to AUC values, flunixin meglumine was more effective than meloxicam 8.57, 4.28, 12.44, and 3.93 times on abomasum, ileum, proximal loop, and centripetal gyri tissues, respectively. ($p<0.001$). As per BPM values, flunixin meglumine was more effective than meloxicam 7.22, 3.88, 7.03, and 3.35 times on abomasum, ileum, proximal loop, and centripetal gyri tissues, respectively. According to P_{MAX} values flunixin meglumine

was more effective than meloxicam 6.13, 4.43, 7.07, and 7.02 times on abomasum, ileum, proximal loop, and centripetal gyri tissues, respectively.

No statistcally significant difference was observed between mean IC_{50} values of AUC, BPM, and P_{MAX} for all tissues in the atropine, flunixin meglumine, and meloxicam group ($p>0.05$) Table 4.3, 4.4, and 4.5. A highly significant difference was observed between mean IC_{50} values of AUC, BPM, and P_{MAX} of atropine, flunixin meglumine, and meloxicam for abomasum, ileum, proximal loop, and centripetal gyri tissues ($p<0.001$) Table 4.4, 4.5 and 4.6.

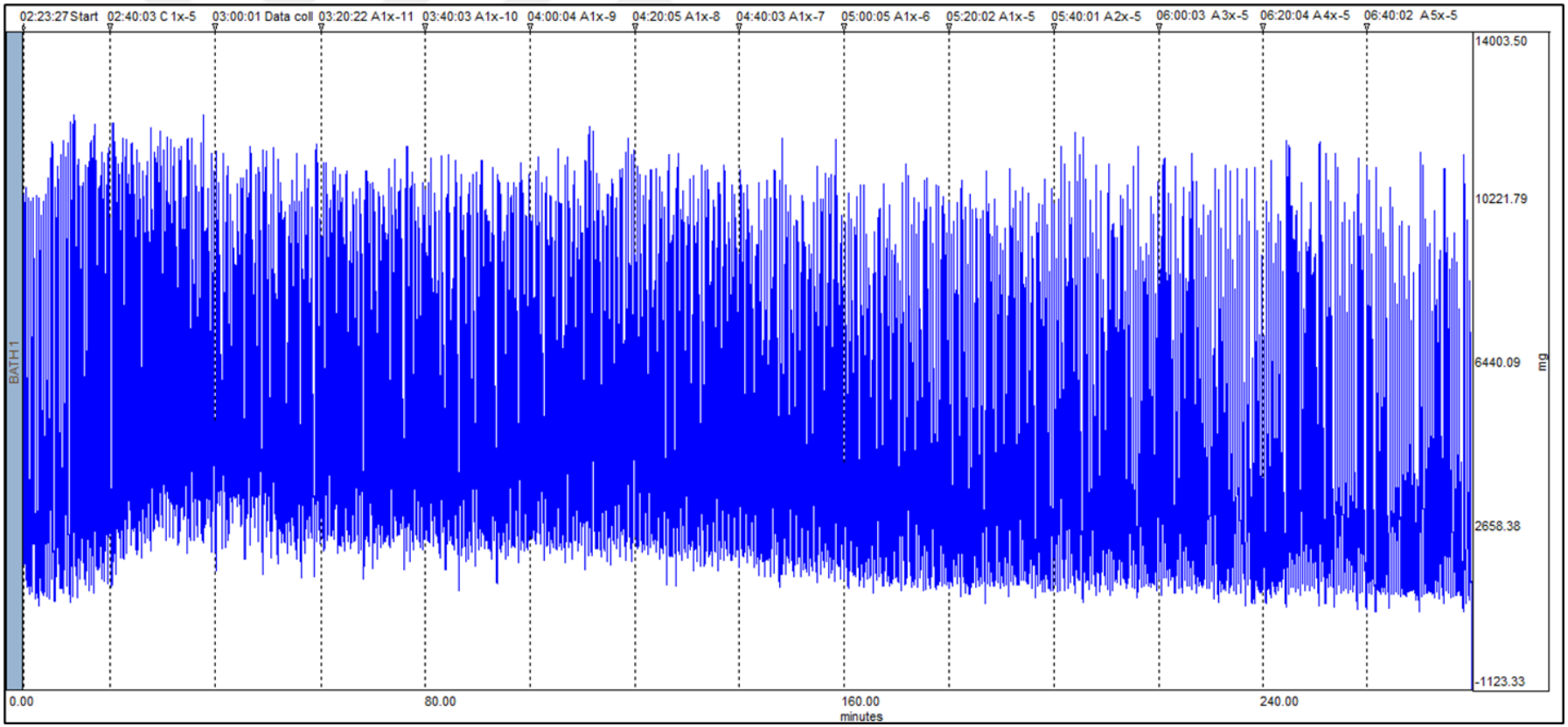

Figure 4.1. Effect of atropine on CH evoked contractions of pyloric antrum of the abomasum

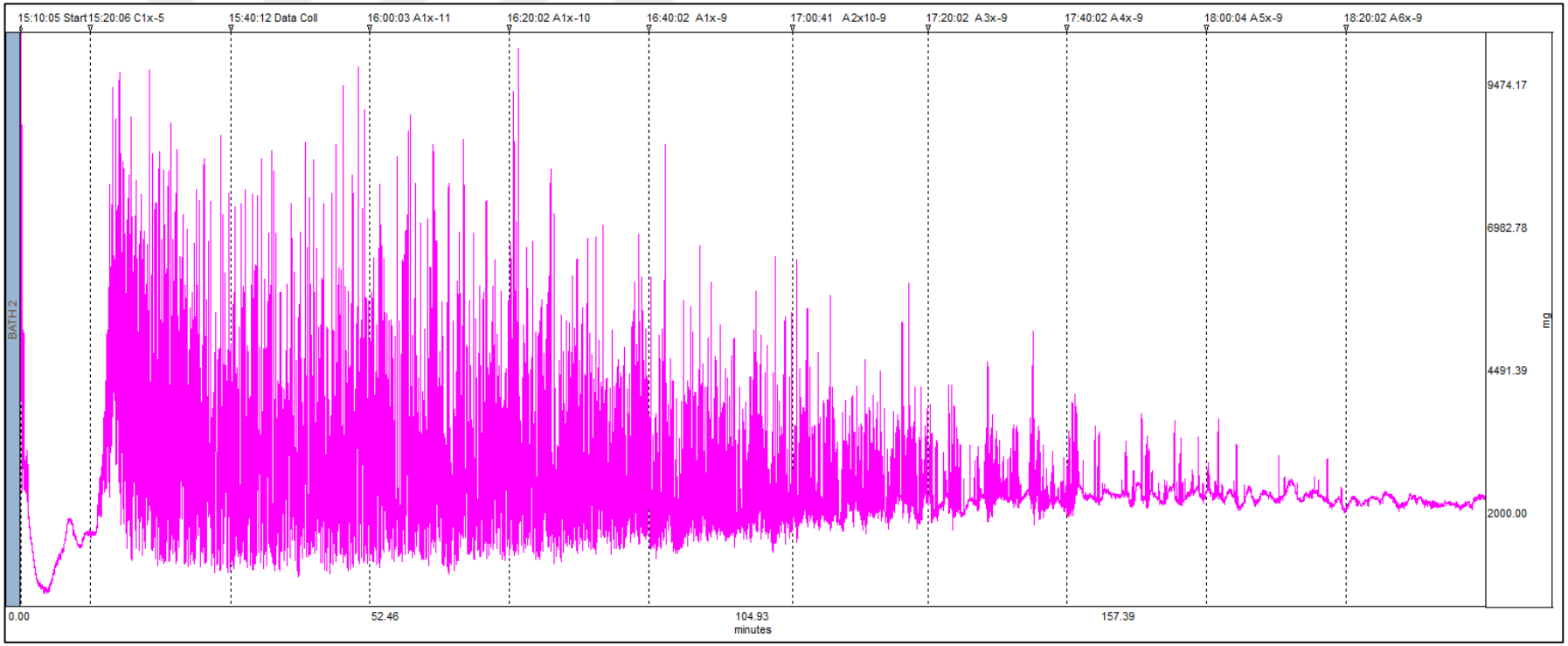

Figure 4.2. Effect of atropine on CH evoked contractions of ileum.

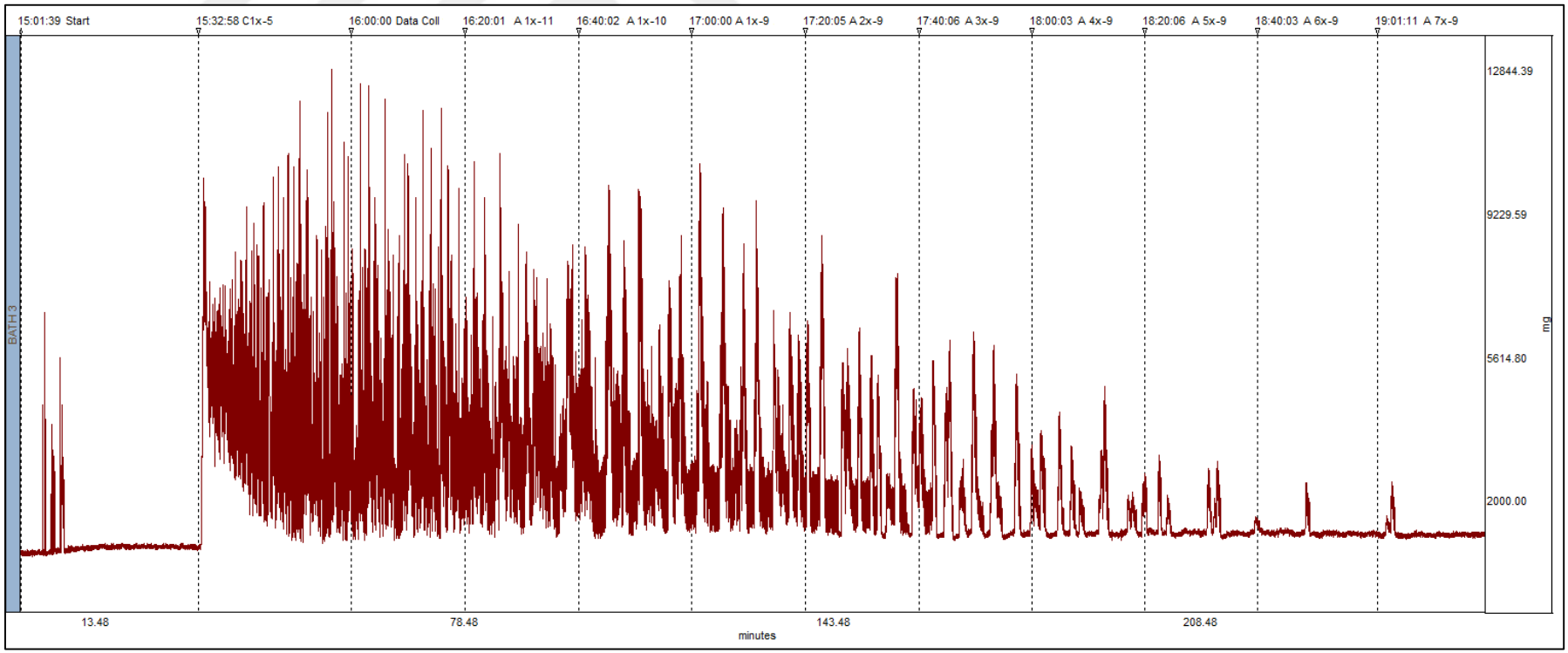

Figure 4.3. Effect of atropine on CH evoked contractions of a proximal loop of ascending colon

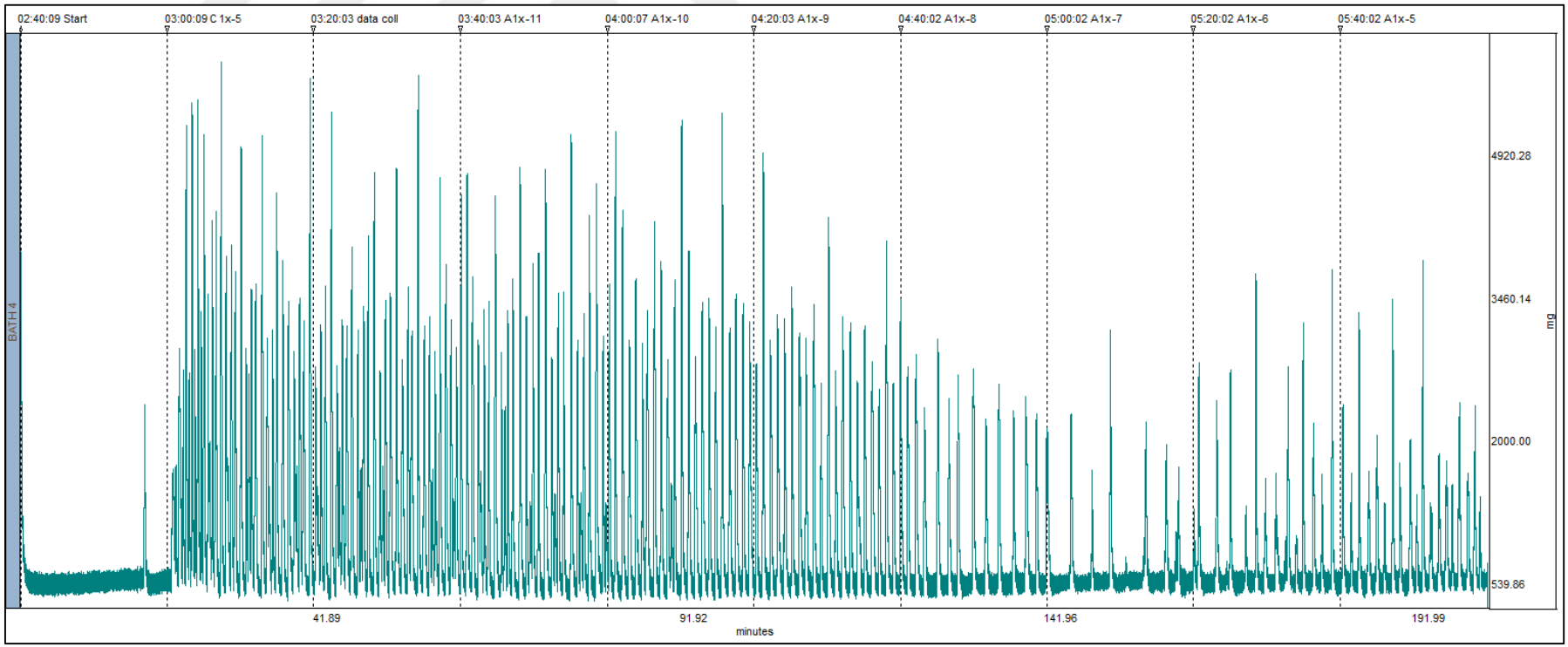

Figure 4.4. Effect of atropine on CH evoked contractions of Centripetal gyri of ascending colon

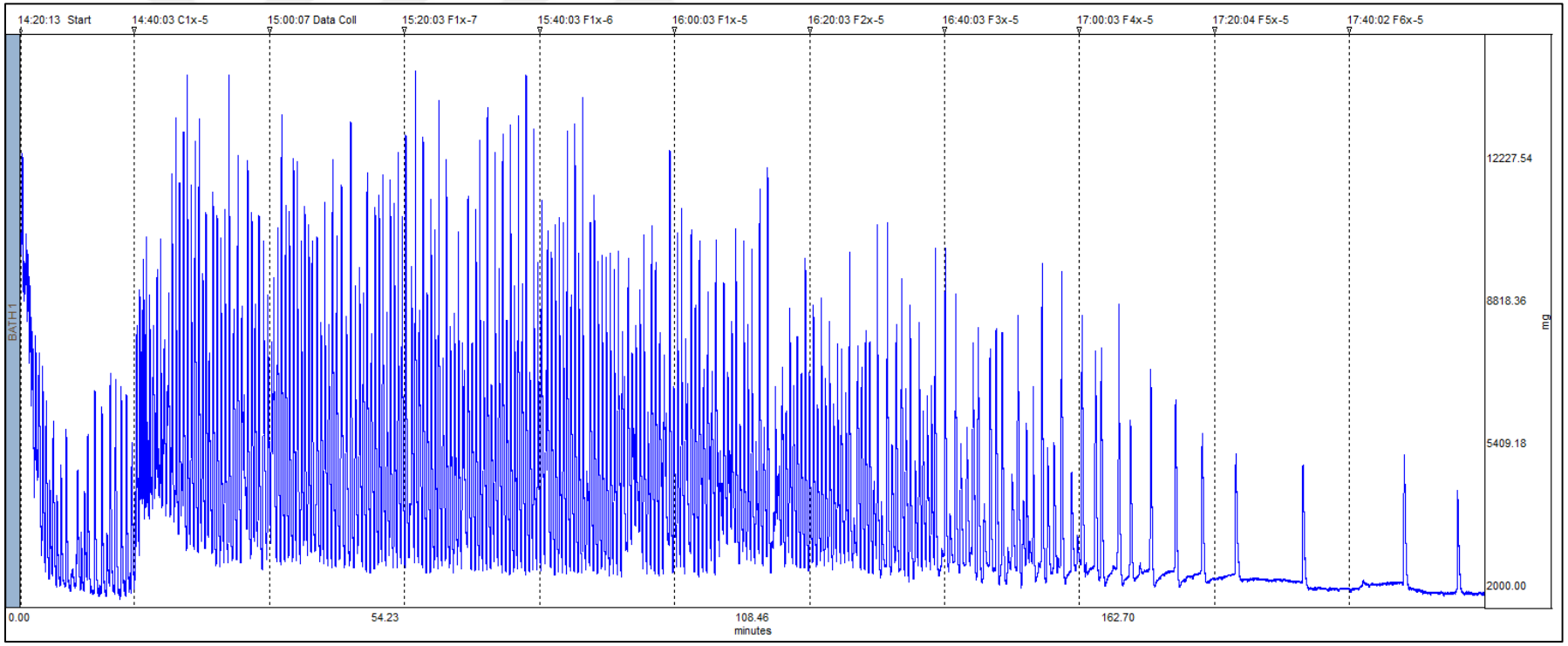

Figure 4.5. Effect of flunixin meglumine on CH evoked contractions of the pyloric antrum of the abomasum

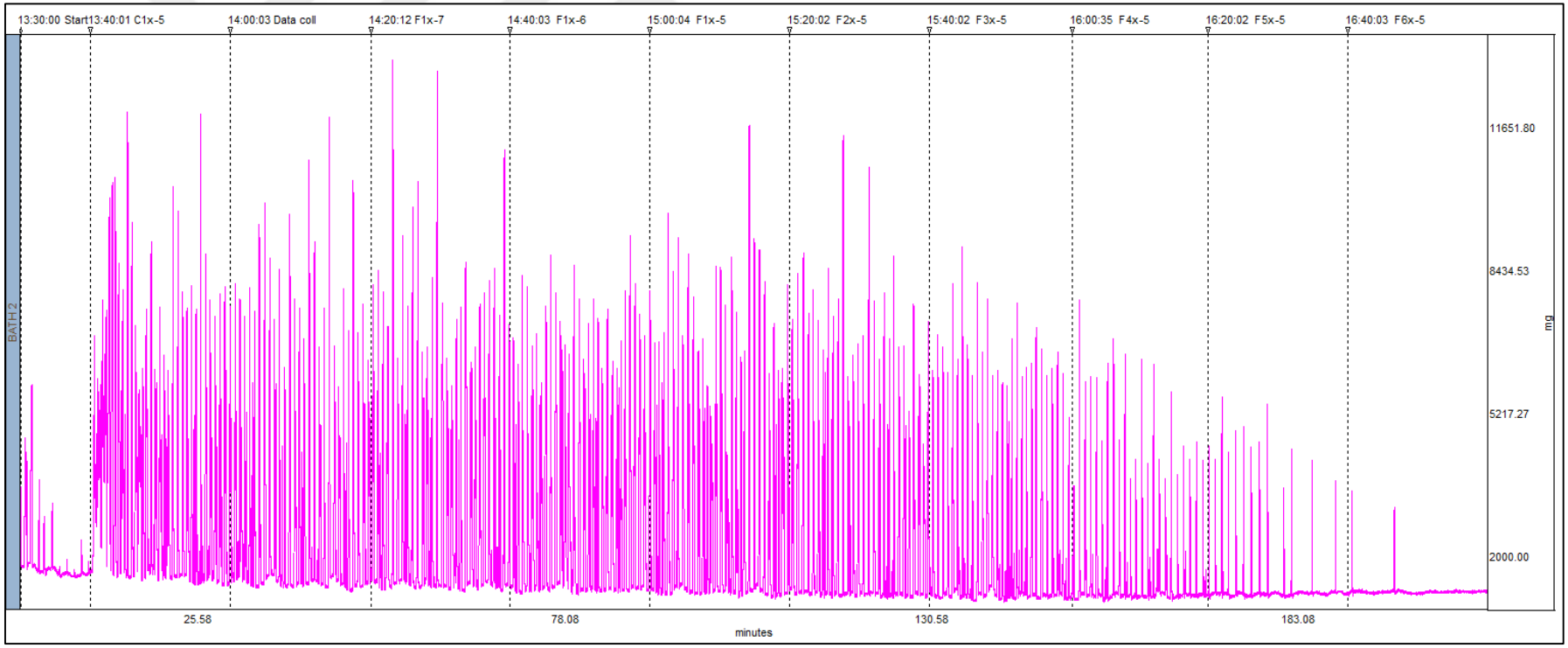

Figure 4.6. Effect of flunixin meglumine on CH evoked contractions of ileum

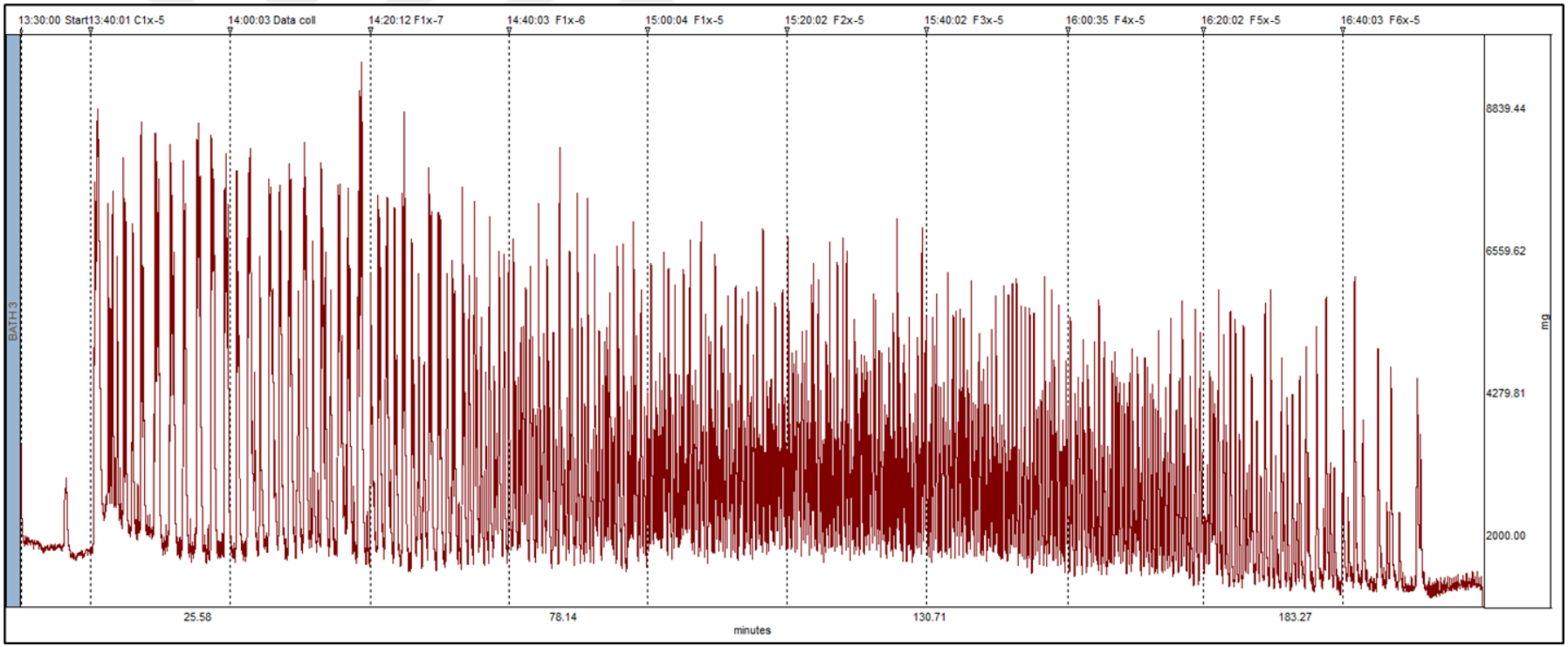

Figure 4.7. Effect of flunixin meglumine on CH evoked contractions of proximal loop of ascending colon

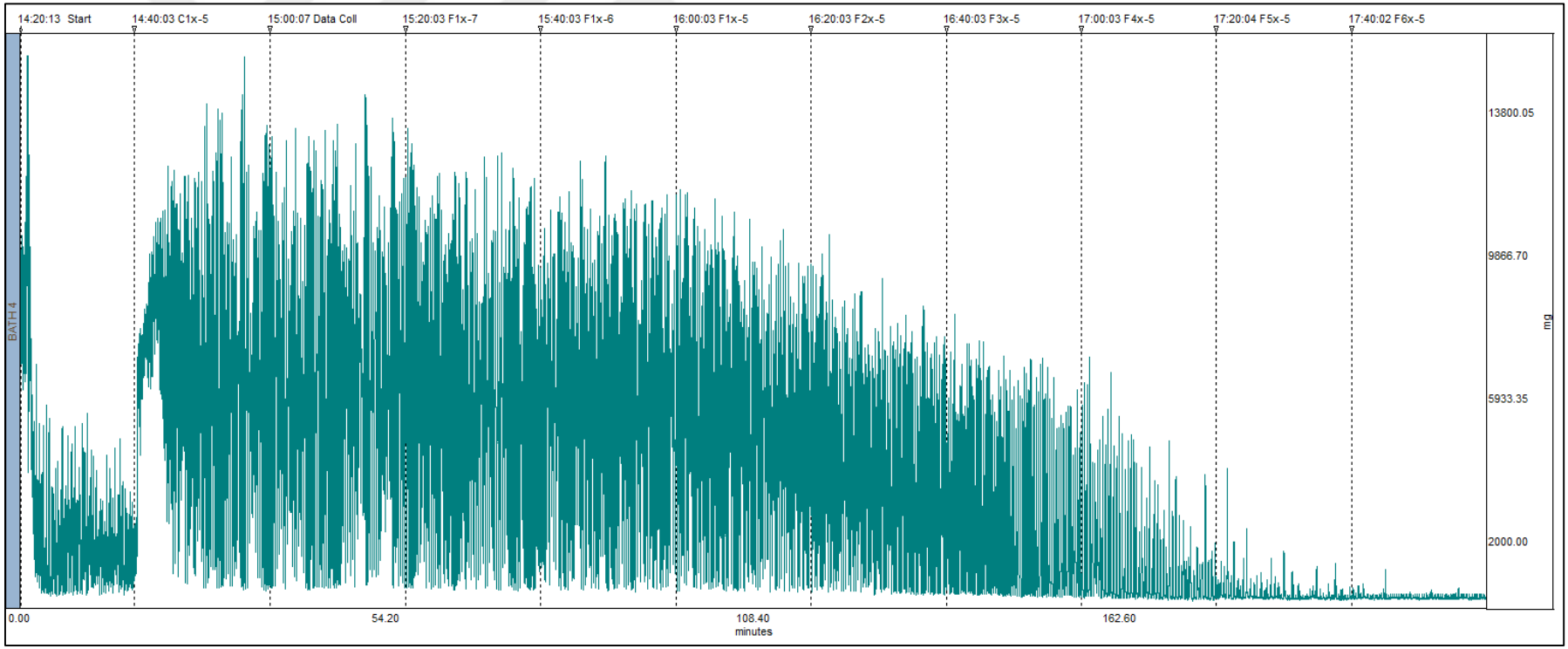

Figure 4.8. Effect of flunixin meglumine on CH evoked contractions of Centripetal gyri of ascending colon

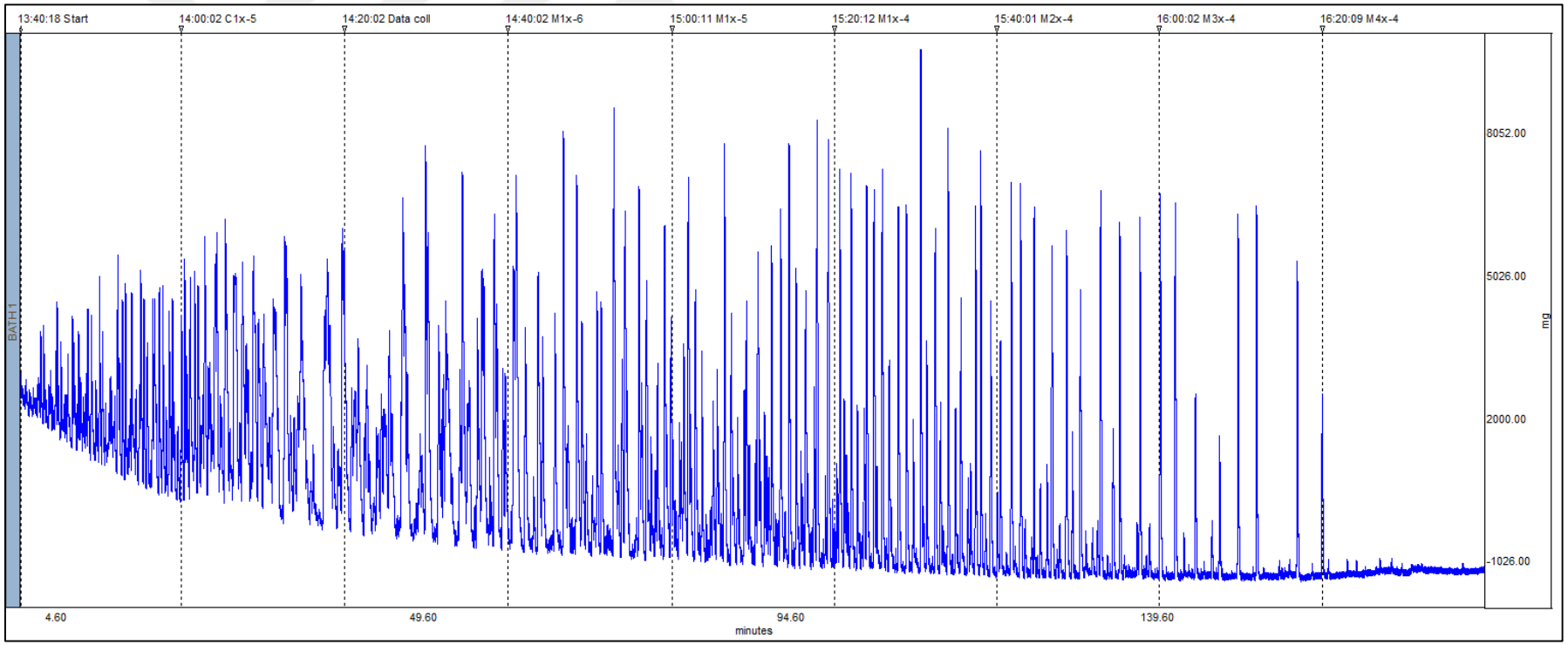

Figure 4.9. Effect of meloxicam on CH evoked contractions of pyloric antrum of the abomasum

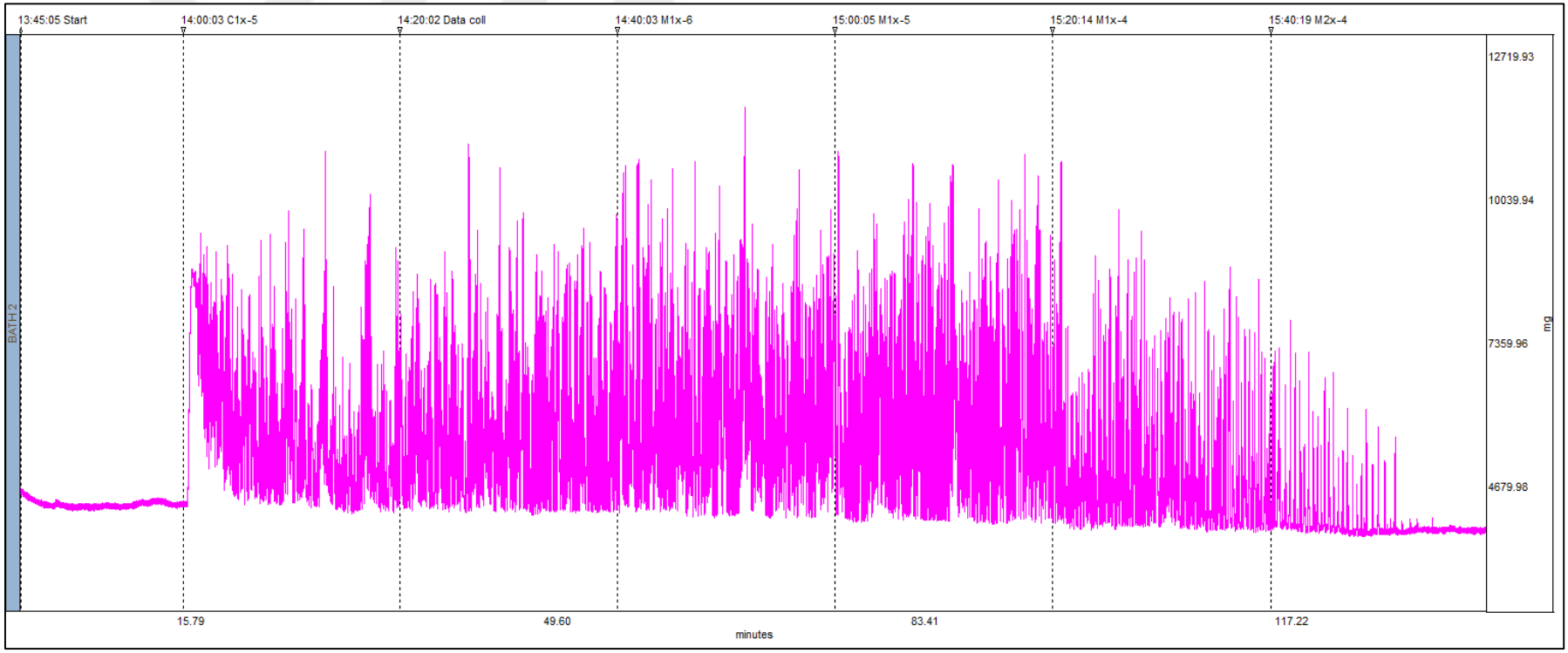

Figure 4.10. Effect of meloxicam on CH evoked contractions of ileum

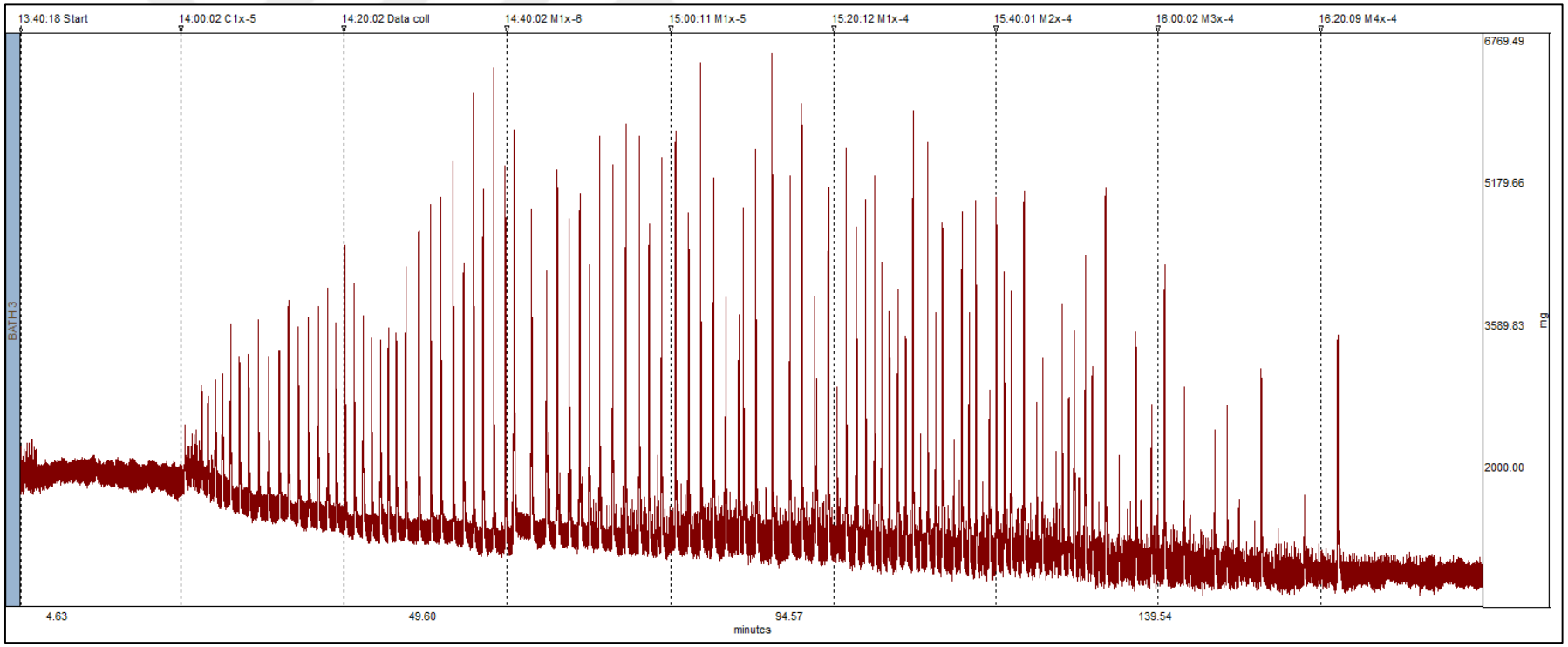

Figure 4.11. Effect of meloxicam on CH evoked contractions of proximal loop of ascending colon

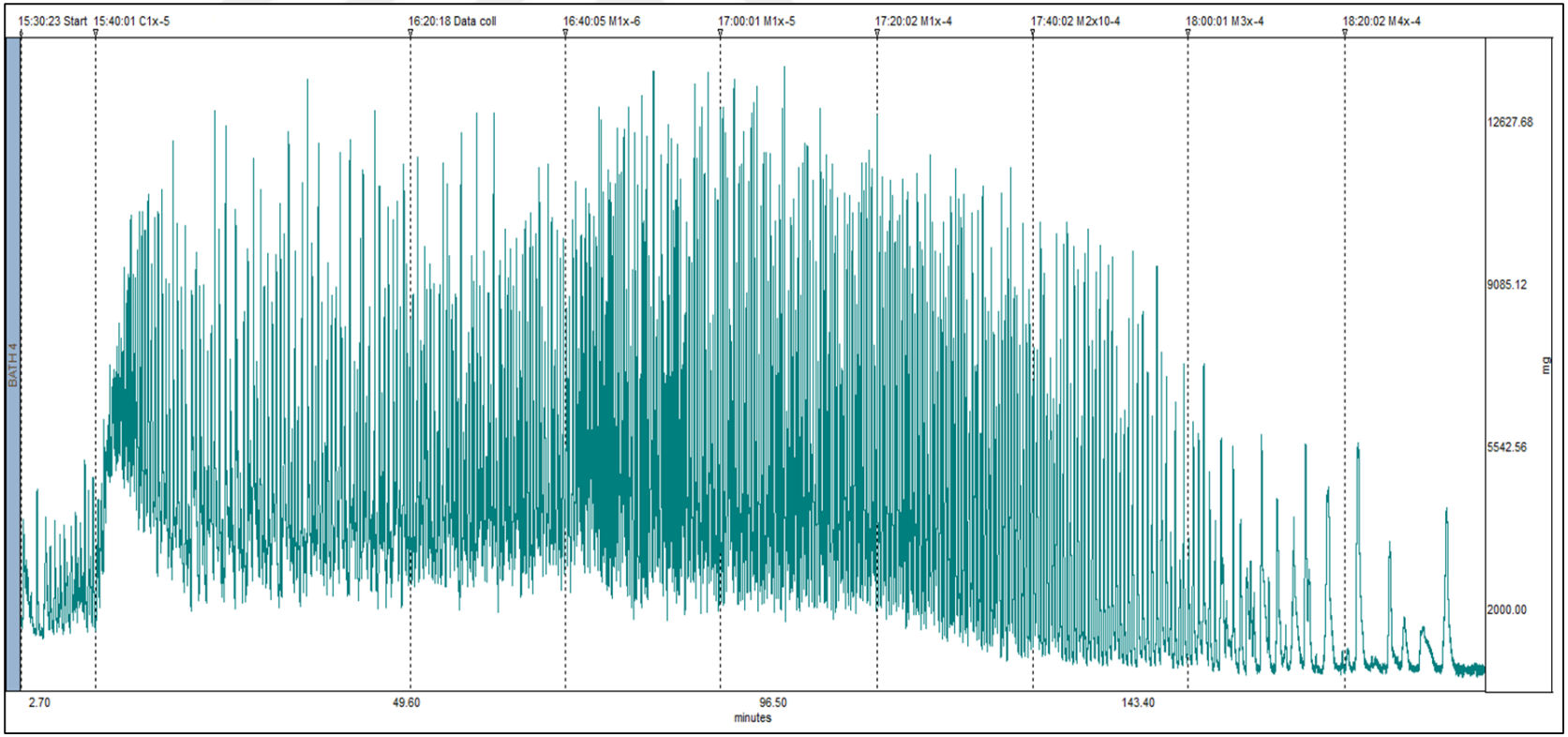

Figure 4.12. Effect of meloxicam on CH evoked contractions of Centripetal gyri of ascending colon

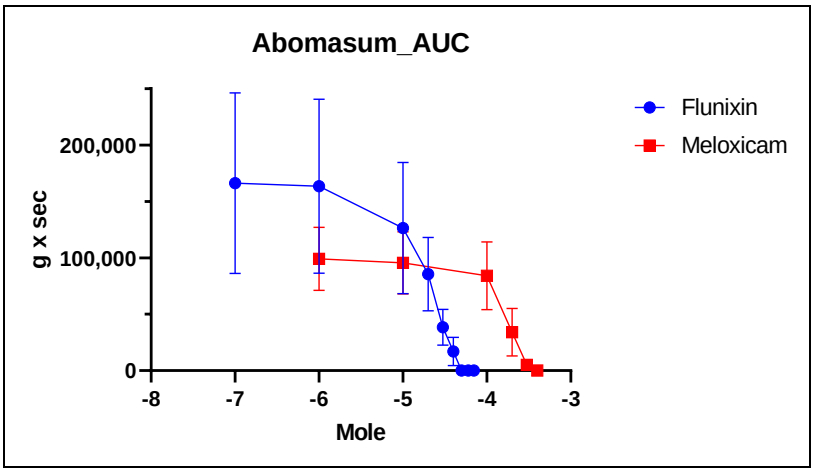

Figure 4.12. Comparison of inhibitory effect of flunixin meglumine and meloxicam on AUC value of abomasum tissue (mean ± SEM)

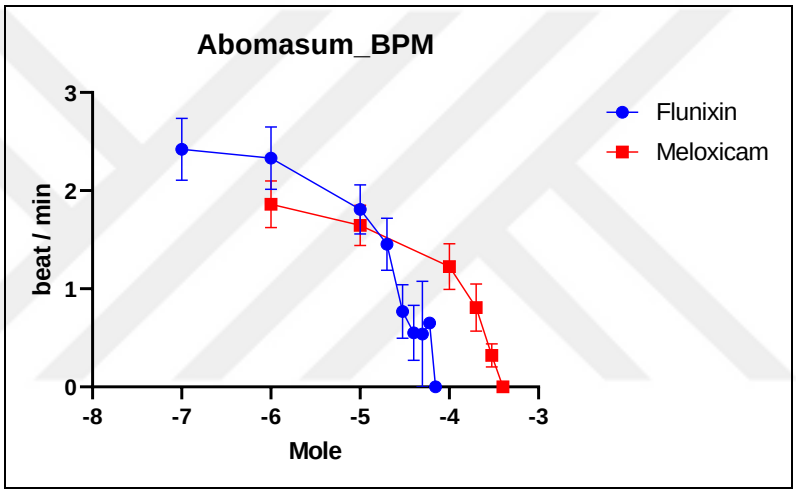

Figure 4.13. Comparison of inhibitory effect of flunixin meglumine and meloxicam on BPM value of abomasum tissue (mean ± SEM)

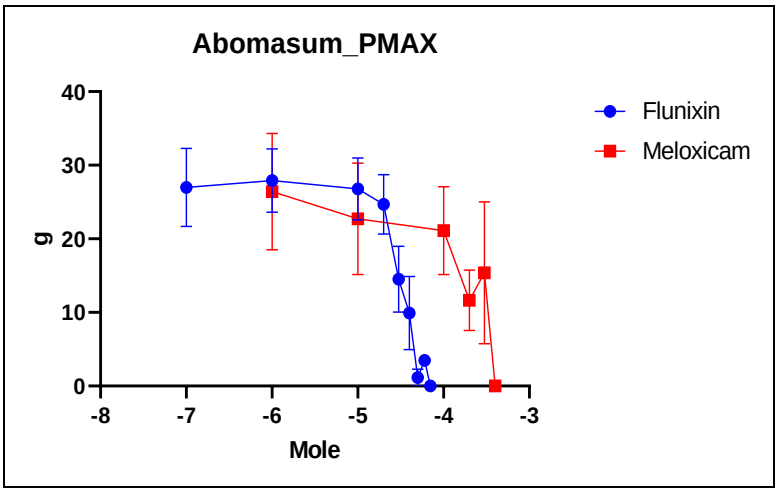

Figure 4.14. Comparison of inhibitory effect of flunixin meglumine and meloxicam on P_{MAX} value of abomasum tissue (mean ± SEM)

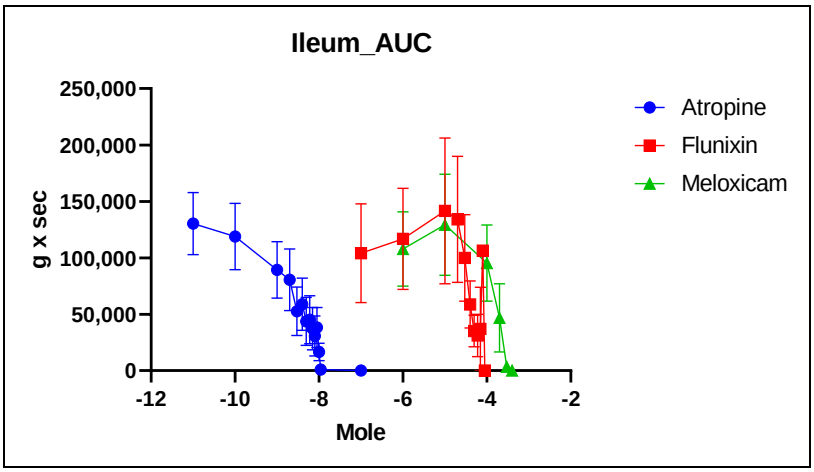

Figure 4.15. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on AUC value of ileum tissue (mean ± SEM)

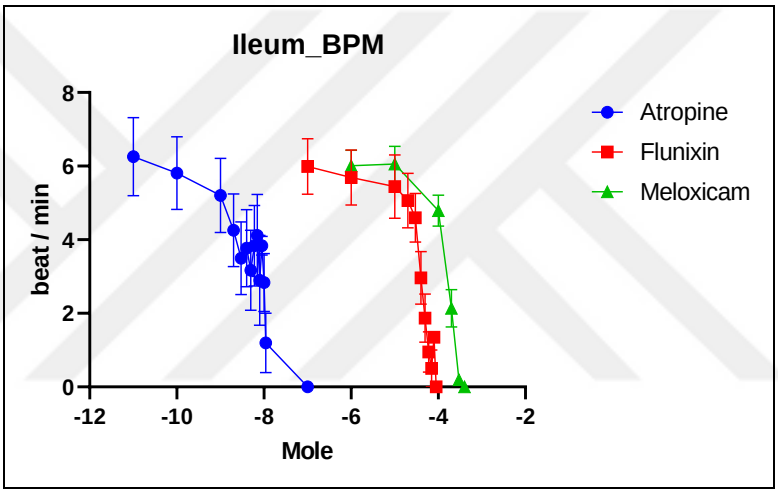

Figure 4.16. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on BPM value of ileum tissue (mean ± SEM)

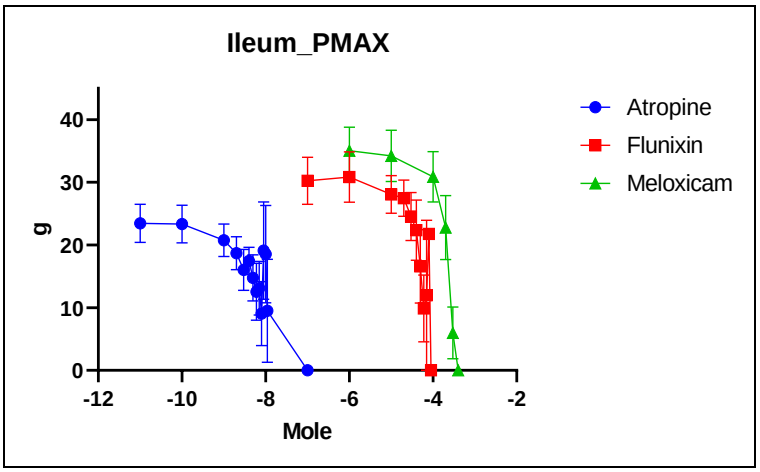

Figure 4.17. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on P_{MAX} value of ileum tissue (mean ± SEM)

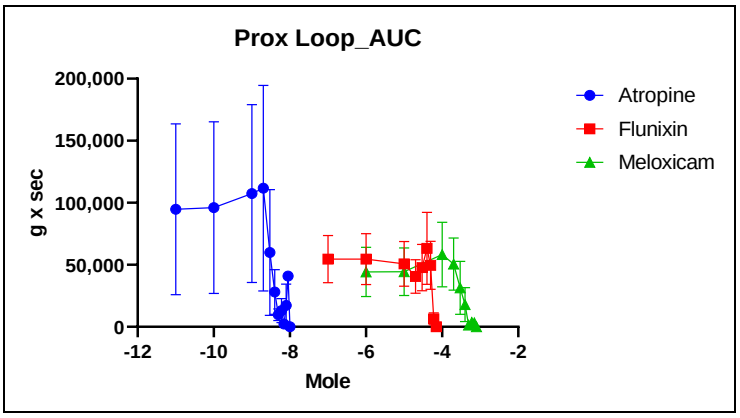

Figure 4.18. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on AUC value of proximal colon tissue (mean ± SEM)

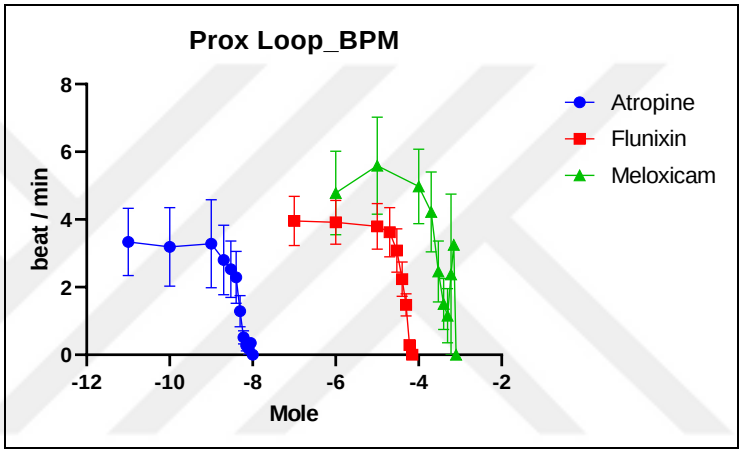

Figure 4.19. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on BPM value of proximal colon tissue (mean ± SEM)

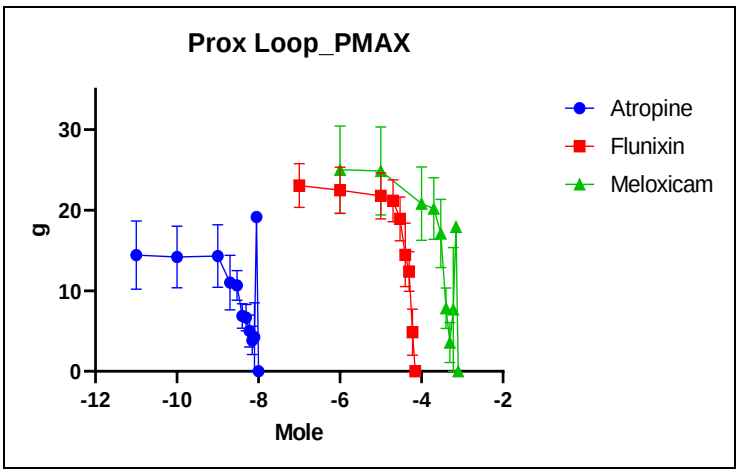

Figure 4.20. Comparison of inhibitory effect of atropine, flunixin meglumine, and meloxicam on P_{MAX} value of proximal colon tissue (mean ± SEM)

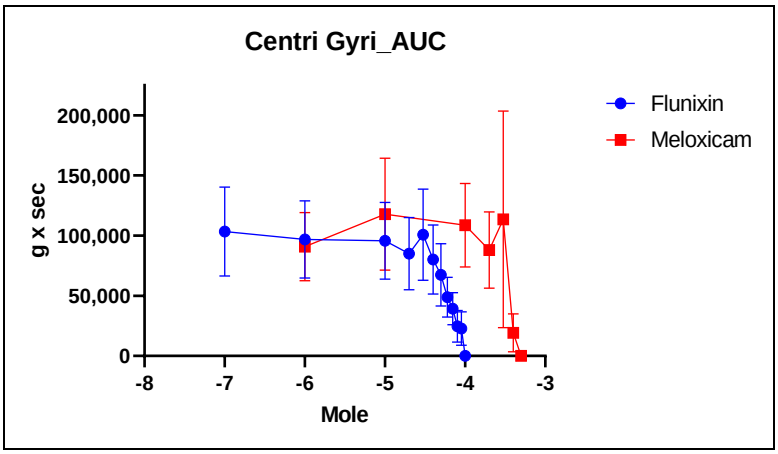

Figure 4.21. Comparison of inhibitory effect of flunixin meglumine, and meloxicam on AUC value of centripetal gyri tissue (mean ± SEM)

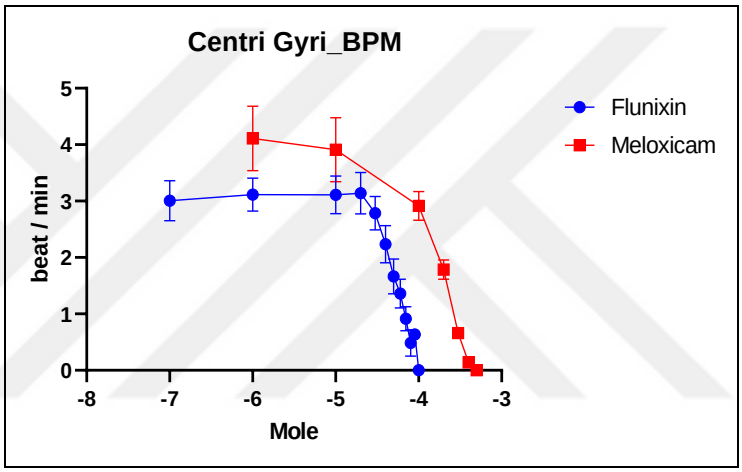

Figure 4.22. Comparison of inhibitory effect of flunixin meglumine, and meloxicam on BPM value of centripetal gyri tissue (mean ± SEM)

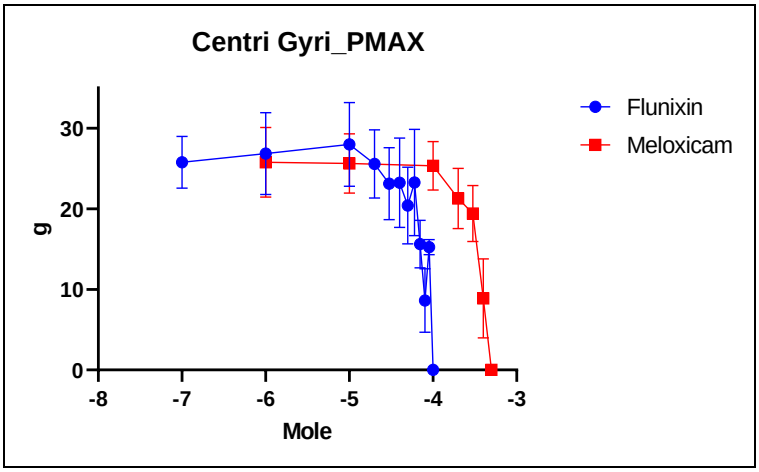

Figure 4.23. Comparison of inhibitory effect of flunixin meglumine, and meloxicam on P_{MAX} value of centripetal gyri tissue (mean ± SEM)

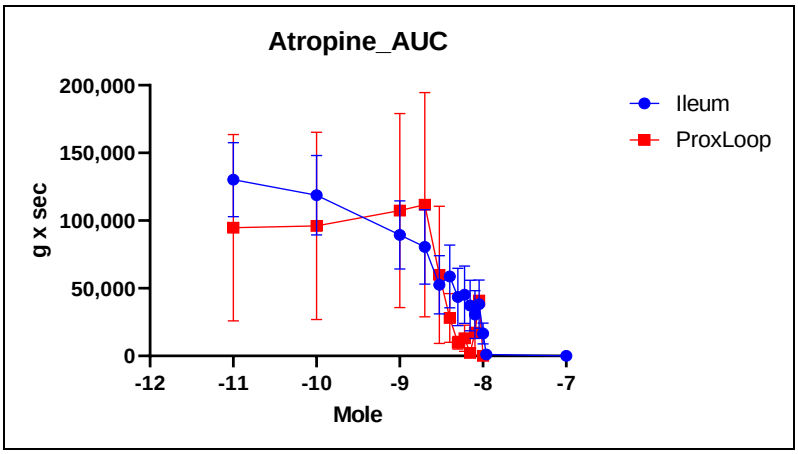

Figure 4.24. Inhibitory effect of atropine on AUC value of ileum and proximal loop (mean ± SEM)

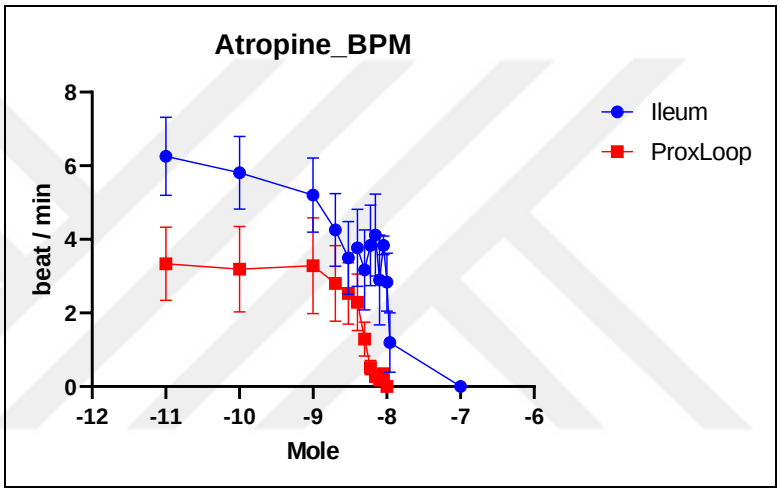

Figure 4.25. Inhibitory effect of atropine on BPM value of ileum and proximal loop (mean ± SEM)

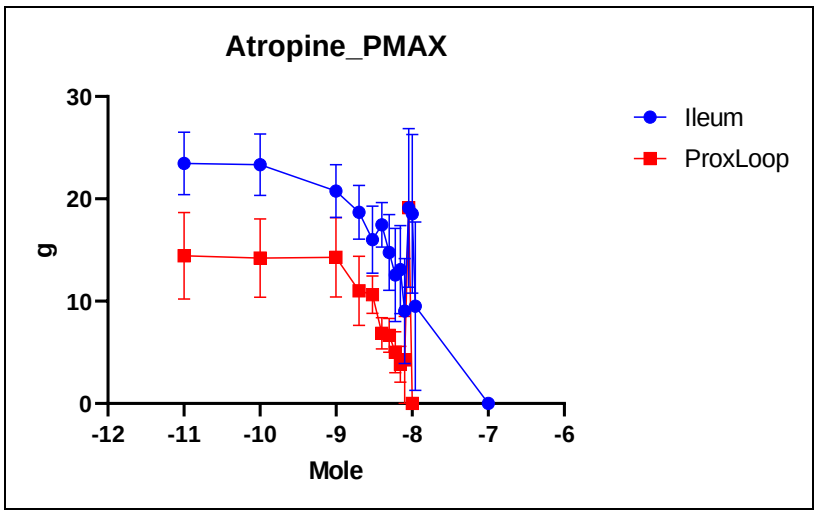

Figure 4.26. Inhibitory effect of atropine on P_{MAX} value of ileum and proximal loop (mean ± SEM)

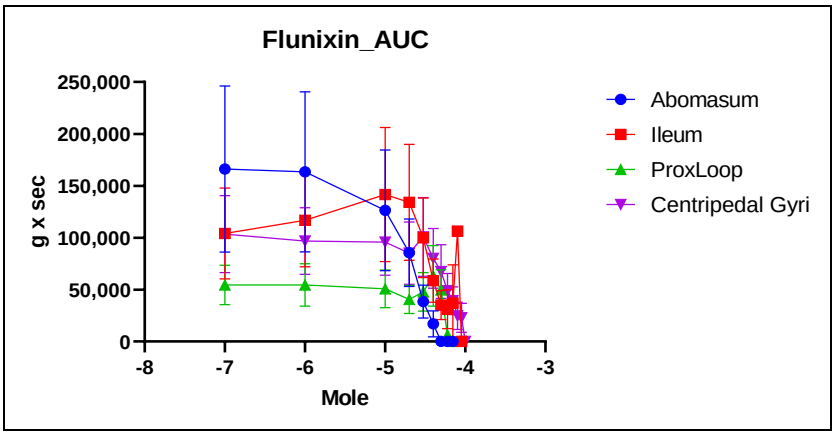

Figure 4.27. Inhibitory effect of flunixin meglumine on AUC value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

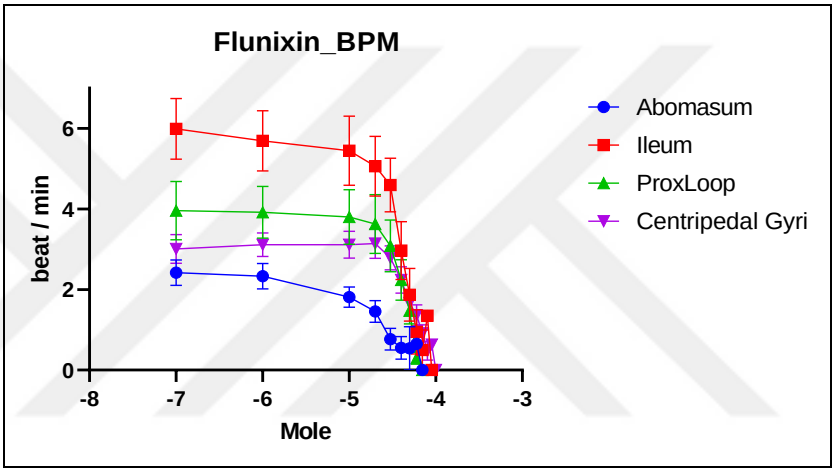

Figure 4.28. Inhibitory effect of flunixin meglumine on BPM value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

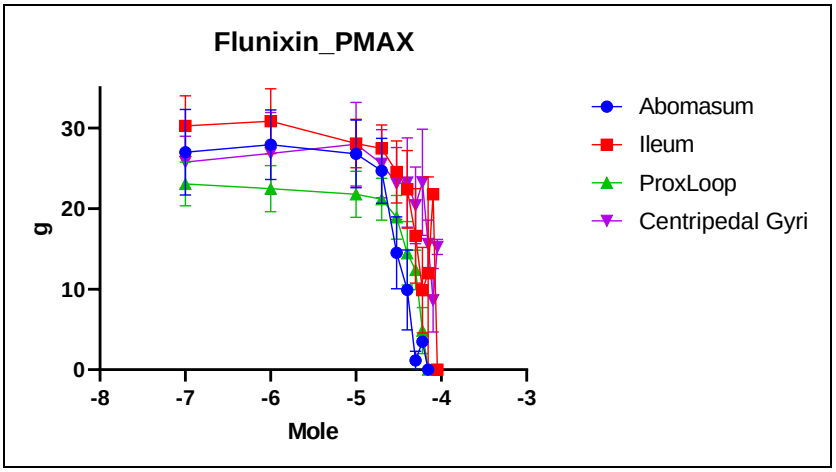

Figure 4.29. Inhibitory effect of flunixin meglumine on P_{MAX} value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

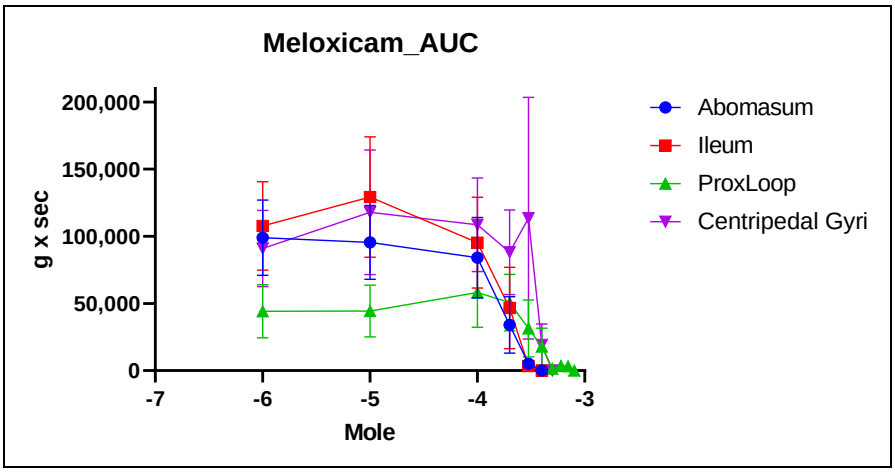

Figure 4.30. Inhibitory effect of meloxicam on AUC value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

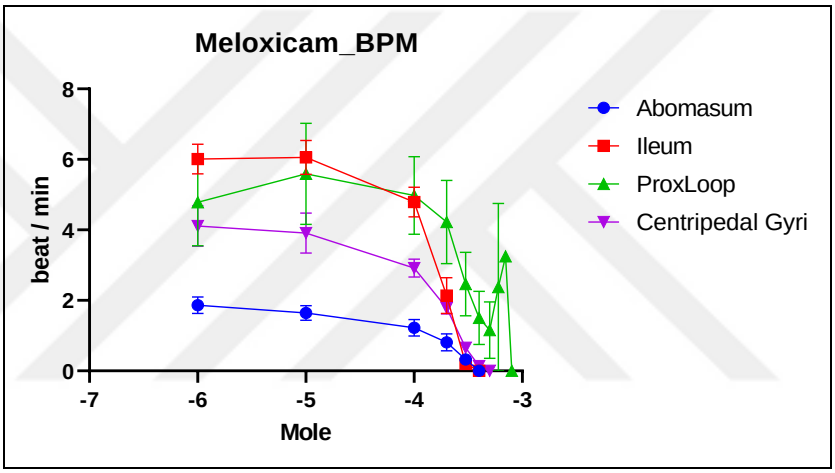

Figure 4.31. Inhibitory effect of meloxicam on BPM value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

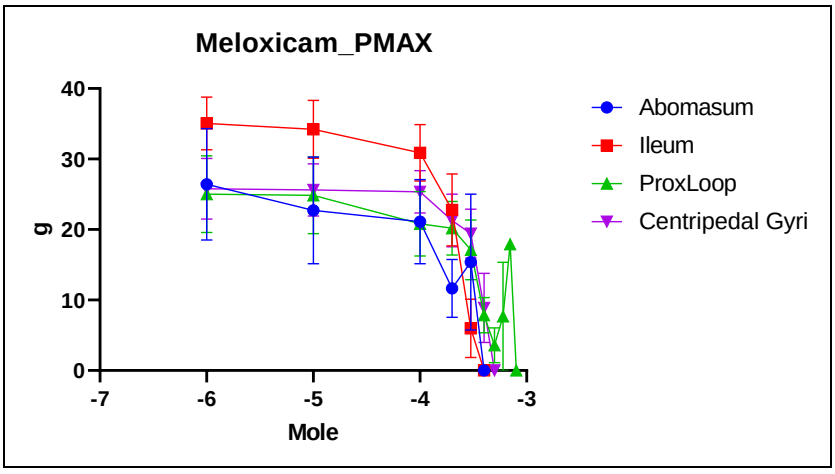

Figure 4.32. Inhibitory effect of meloxicam on P_{MAX} value of abomasum, ileum, proximal loop and centripetal gyri (mean ± SEM)

Table 4.1. IC_{50} values (Mole) of atropine, flunixin meglumine and meloxicam for AUC, BPM, and P_{MAX}

	ATR			FLU			MELOX		
	AUC	BPM	P_{MAX}	AUC	BPM	P_{MAX}	AUC	BPM	P_{MAX}
Abomasum				$1.97x10^{-5}$	$2.37x10^{-5}$	$3.13x10^{-5}$	$1.69x10^{-4}$	$1.71x10^{-4}$	$1.92x10^{-4}$
95 % CLM				$1.71x10^{-5}$	$2.01x10^{-5}$	$2.98x10^{-5}$	$1.56x10^{-4}$	$1.27x10^{-4}$	$1.34x10^{-4}$
				$2.25x10^{-5}$	$2.85x10^{-5}$	$3.28x10^{-5}$	$1.84x10^{-4}$	$2.49x10^{-4}$	$2.53x10^{-4}$
Ileum	$2.82x10^{-9}$	$4.15x10^{-9}$	$4.66x10^{-9}$	$3.95 x10^{-5}$	$4.15x10^{-5}$	$5.01x10^{-5}$	$1.69x10^{-4}$	$1.61x10^{-4}$	$2.22x10^{-4}$
95 % CLM	$2.17x10^{-9}$	$3.31x10^{-9}$	$3.94x10^{-9}$	$3.39x10^{-5}$	$3.88x10^{-5}$	$4.60x10^{-5}$	$1.32x10^{-4}$	$1.42x10^{-4}$	$2.07x10^{-4}$
	$3.59x10^{-9}$	$5.11x10^{-9}$	$5.47x10^{-9}$	$4.76x10^{-5}$	$4.46x10^{-5}$	$5.68x10^{-5}$	$2.09x10^{-4}$	$1.82x10^{-4}$	$2.36x10^{-4}$
Prox. Loop	$3.26x10^{-9}$	$4.63x10^{-9}$	$4.40x10^{-9}$	$2.75x10^{-5}$	$4.24x10^{-5}$	$4.67x10^{-5}$	$3.42x10^{-4}$	$2.98x10^{-4}$	$3.30x10^{-4}$
95 % CLM	$2.97x10^{-9}$	$4.24x10^{-9}$	$3.71x10^{-9}$	$2.12x10^{-5}$	$3.98x10^{-5}$	$4.35x10^{-5}$	$2.85x10^{-4}$	$2.60x10^{-4}$	$2.85x10^{-4}$
	$3.59x10^{-9}$	$5.00x10^{-9}$	$5.15x10^{-9}$	$4.84x10^{-5}$	$4.50x10^{-5}$	$5.03x10^{-5}$	$4.18x10^{-4}$	$3.57x10^{-4}$	$3.69x10^{-4}$
Centri. Gyri				$5.78x10^{-5}$	$5.23x10^{-5}$	$4.93x10^{-5}$	$2.27x10^{-4}$	$1.75x10^{-4}$	$3.46x10^{-4}$
95 % CLM				$5.14x10^{-5}$	$4.93x10^{-5}$	$4.20x10^{-5}$	$1.81x10^{-4}$	$1.47x10^{-4}$	$3.14x10^{-4}$
				$7.69x10^{-5}$	$5.63x10^{-5}$	$6.50x10^{-5}$	$2.74x10^{-4}$	$2.09x10^{-4}$	$3.75x10^{-4}$

Table 4.2. Ratio of IC_{50} values of meloxicam/flunixin meglumine

	AUC	BPM	P_{MAX}
Abomasum	8.57	7.22	6.13
İleum	4.28	3.88	4.43
Proximal Loop	12.44	7.03	7.07
Centripetal Gyri	3.93	3.35	7.02

Table 4.3. Comparison of IC_{50} of AUC, BPM and P_{MAX} values according to tissues in the atropine group (mole)

Tissue		AUC	BPM	P_{MAX}
Ileum (mean)		$2.82x10^{-9}$	$4.15x10^{-9}$	$4.66x10^{-9}$
(95%CLM)		$2.17x10^{-9}$ $3.59x10^{-9}$	$3.31x10^{-9}$ $5.11x10^{-9}$	$3.94x10^{-9}$ $5.47x10^{-9}$
Proximal loop (mean)		$3.26x10^{-9}$	$4.63x10^{-9}$	$4.40x10^{-9}$
(95%CLM)		$2.97x10^{-9}$ $3.59x10^{-9}$	$4.24x10^{-9}$ $5.00x10^{-9}$	$3.71x10^{-9}$ $5.15x10^{-9}$
Test statistic	F	1.253	1.136	0.2852
	P	0.2762	0.2993	0.5992

In the atropine group, the mean IC50 values for the AUC, BPM and P_{MAX} of ileum and proximal tissues were compared. There was no statistically significant difference found between mean AUC, BPM and P_{MAX} values of ileum and proximal loop tissues ($p>0.05$).

Table 4.4. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to tissues in the flunixin meglumine group (mole)

Tissue		AUC	BPM	P_{MAX}
Abomasum (mean)		$1.97x10^{-5}$	$2.37x10^{-5}$	$3.13x10^{-5}$
(95%CLM)		$1.71x10^{-5}$ $2.25x10^{-5}$	$2.01x10^{-5}$ $2.85x10^{-5}$	$2.98x10^{-5}$ $3.28x10^{-5}$
Ileum (mean)		$3.95\ x10^{-5}$	$4.15x10^{-5}$	$5.01x10^{-5}$
(95%CLM)		$3.39x10^{-5}$ $4.76x10^{-5}$	$3.88x10^{-5}$ $4.46x10^{-5}$	$4.60x10^{-5}$ $5.68x10^{-5}$
Proximal loop (mean)		$2.75x10^{-5}$	$4.24x10^{-5}$	$4.67x10^{-5}$
(95%CLM)		$2.12x10^{-5}$ $4.84x10^{-5}$	$3.98x10^{-5}$ $4.50x10^{-5}$	$4.35x10^{-5}$ $5.03x10^{-5}$
Centripetal Gyri (mean)		$5.78x10^{-5}$	$5.23x10^{-5}$	$4.93x10^{-5}$
(95%CLM)		$5.14x10^{-5}$ $7.69x10^{-5}$	$4.93x10^{-5}$ $5.63x10^{-5}$	$4.20x10^{-5}$ $6.50x10^{-5}$
Test statistic	F	2.704	1.852	0.7574
	P	0.0670	0.1636	0.5286

In the flunixin meglumine group, the mean IC_{50} values for the AUC, BPM and P_{MAX} of abomasum, ileum, proximal loop, and centripetal gyri were compared. There was no statistically significant difference found between the mean of three parameters ($p>0.05$).

Table 4.5. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to tissues in the meloxicam group (mole)

Tissue		AUC	BPM	P_{MAX}
Abomasum (mean)		$1.69x10^{-4}$	$1.71x10^{-4}$	$1.92x10^{-4}$
(95%CLM)		$1.56x10^{-4}$ $1.84x10^{-4}$	$1.27x10^{-4}$ $2.49x10^{-4}$	$1.34x10^{-4}$ $2.53x10^{-4}$
Ileum (mean)		$1.69x10^{-4}$	$1.61x10^{-4}$	$2.22x10^{-4}$
(95%CLM)		$1.32x10^{-4}$ $2.09x10^{-4}$	$1.42x10^{-4}$ $1.82x10^{-4}$	$2.07x10^{-4}$ $2.36x10^{-4}$
Proximal loop (mean)		$3.42x10^{-4}$	$2.98x10^{-4}$	$3.30x10^{-4}$
(95%CLM)		$2.85x10^{-4}$ $4.18x10^{-4}$	$2.60x10^{-4}$ $3.57x10^{-4}$	$2.85x10^{-4}$ $3.69x10^{-4}$
Centripetal Gyri (mean)		$2.27x10^{-4}$	$1.75x10^{-4}$	$3.46x10^{-4}$
(95%CLM)		$1.81x10^{-4}$ $2.74x10^{-4}$	$1.47x10^{-4}$ $2.09x10^{-4}$	$3.14x10^{-4}$ $3.75x10^{-4}$
Test statistic	F	2.087	0.2971	0.6263
	P	0.1515	0.8269	0.6107

In the meloxicam group, the mean IC_{50} values for the AUC, BPM and P_{MAX} of abomasum, ileum, proximal loop, and centripetal gyri were compared. There was no statistically significant difference found between the mean of three parameters ($p>0.05$).

Table 4.6. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to the drug in abomasum tissue (mole)

Tissue		AUC	BPM	P_{MAX}
Flunixin meglumine (mean)		$1.97x10^{-5}$	$2.37x10^{-5}$	$3.13x10^{-5}$
(95%CLM)		$1.71x10^{-5}$ $2.25x10^{-5}$	$2.01x10^{-5}$ $2.85x10^{-5}$	$2.98x10^{-5}$ $3.28x10^{-5}$
Meloxicam (mean)		$1.69x10^{-4}$	$1.71x10^{-4}$	$1.92x10^{-4}$
(95%CLM)		$1.56x10^{-4}$ $1.84x10^{-4}$	$1.27x10^{-4}$ $2.49x10^{-4}$	$1.34x10^{-4}$ $2.53x10^{-4}$
Test statistic	F	146.7	77.60	92.38
	P	**<0.0001**	**<0.0001**	**<0.0001**

In abomasum, the mean IC_{50} values of AUC, BPM and P_{MAX} for flunixin meglumine and meloxicam were compared. A highly statistically significant difference was observed between IC_{50} values of two drugs for all three parameters.

Table 4.7. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to drug in the ileum tissue (mole)

Tissue		AUC	BPM	P_{MAX}
Atropine (mean)		$2.82x10^{-9}$	$4.15x10^{-9}$	$4.66x10^{-9}$
(95%CLM)		$2.17x10^{-9}$ $3.59x10^{-9}$	$3.31x10^{-9}$ $5.11x10^{-9}$	$3.94x10^{-9}$ $5.47x10^{-9}$
Flunixin meglumine (mean)		$3.95\ x10^{-5}$	$4.15x10^{-5}$	$5.01x10^{-5}$
(95%CLM)		$3.39x10^{-5}$ $4.76x10^{-5}$	$3.88x10^{-5}$ $4.46x10^{-5}$	$4.60x10^{-5}$ $5.68x10^{-5}$
Meloxicam (mean)		$1.69x10^{-4}$	$1.61x10^{-4}$	$2.22x10^{-4}$
(95%CLM)		$1.32x10^{-4}$ $2.09x10^{-4}$	$1.42x10^{-4}$ $1.82x10^{-4}$	$2.07x10^{-4}$ $2.36x10^{-4}$
Test statistic	F	288.2	645.9	707.8
	P	**<0.0001**	**<0.0001**	**<0.0001**

In ileum, the mean IC_{50} values of AUC, BPM and P_{MAX} for atropine, flunixin meglumine and meloxicam were compared. A statistically significant difference was found between IC_{50} values of three drugs for all three parameters.

Table 4.8. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to drug in the proximal loop tissue (mole)

Tissue		AUC	BPM	P_{MAX}
Atropine (mean)		$3.26x10^{-9}$	$4.63x10^{-9}$	$4.40x10^{-9}$
(95%CLM)		$2.97x10^{-9}$ $3.59x10^{-9}$	$4.24x10^{-9}$ $5.00x10^{-9}$	$3.71x10^{-9}$ $5.15x10^{-9}$
Flunixin meglumine (mean)		$2.75x10^{-5}$	$4.24x10^{-5}$	$4.67x10^{-5}$
(95%CLM)		$2.12x10^{-5}$ $4.84x10^{-5}$	$3.98x10^{-5}$ $4.50x10^{-5}$	$4.35x10^{-5}$ $5.03x10^{-5}$
Meloxicam (mean)		$3.42x10^{-4}$	$2.98x10^{-4}$	$3.30x10^{-4}$
(95%CLM)		$2.85x10^{-4}$ $4.18x10^{-4}$	$2.60x10^{-4}$ $3.57x10^{-4}$	$2.85x10^{-4}$ $3.69x10^{-4}$
Test statistic	F	374.1	919	523.3
	P	**<0.0001**	**<0.0001**	**<0.0001**

In proximal loop, the mean IC_{50} values of AUC, BPM and P_{MAX} for atropine, flunixin meglumine and meloxicam were compared. A statistically significant difference was found between IC_{50} values of all three drugs for three parameters.

Table 4.9. Comparison of IC_{50} of AUC, BPM, and P_{MAX} values according to drug in the centripetal gyri tissue (mole)

Tissue		AUC	BPM	P_{MAX}
Flunixin meglumine (mean)		$5.78x10^{-5}$	$5.23x10^{-5}$	$4.93x10^{-5}$
(95%CLM)		$5.14x10^{-5}$ $7.69x10^{-5}$	$4.93x10^{-5}$ $5.63x10^{-5}$	$4.20x10^{-5}$ $6.50x10^{-5}$
Meloxicam (mean)		$2.27x10^{-4}$	$1.75x10^{-4}$	$3.46x10^{-4}$
(95%CLM)		$1.81x10^{-4}$ $2.74x10^{-4}$	$1.47x10^{-4}$ $2.09x10^{-4}$	$3.14x10^{-4}$ $3.75x10^{-4}$
Test statistic	F	130.2	232.6	128.3
	P	**<0.0001**	**<0.0001**	**<0.0001**

In centripetal gyri, the mean IC_{50} values of AUC, BPM and P_{MAX} for flunixin meglumine and meloxicam were compared. A highly statistically significant difference was observed between IC_{50} values of two drugs for all three parameters.

4.2. DISCUSSION

NSAIDs have been extensively used in veterinary medicine for acute and chronic pain due to musculoskeletal disorders, mastitis, soft tissue injuries, postoperative pain, etc. NSAIDs exert their antipyretic, anti-inflammatory, and analgesic effects by impeding the action of PG synthesis via inhibition of COX enzyme (Akın and Karademir, 2017). Use of NSAIDs as pain killer effects the smooth muscle activity of GIT. NSAIDs exert their excitatory and inhibitory effect according to the concentration of a drug, type of species, part of GIT, and smooth muscle tissue preparations. In *vivo* administration of flunixin meglumine at dose of 2 mg/kg and meloxicam at a dose of 0.5 mg/kg is recommended as part of the initial treatment in animals suffering from hypermotility. The advantageous effects of both drugs could be caused by their analgesic, anti-inflammatory, antipyretic, or antisecretory properties (Constable, 2009).

Since no data is available to show the direct and comparative effect of flunixin meglumine and meloxicam on different parts of small and large intestine in cattle. In this study, we investigated the comparative inhibitory effect of both drugs. This inhibitory action of flunixin meglumine and meloxicam on GIT preparations possibly allocate the inhibition of PG synthesis through their action on COX enzyme.

From the IC_{50} values of the two NSAIDs tested, flunixin had the strongest anti-COX-1 activity while meloxicam had the strongest anti-COX-2 activity. The IC_{50} values of meloxicam compared with flunixin meglumine specified that the meloxicam is a less potent inhibitor of COX-1 with lesser adverse GI effects when used in therapy.

Menozzi et al. (2009) investigated the effect of flunixin meglumine in isolated horse ileum preparations. They concluded that flunixin meglumine at a higher concentration of 1-100 μM inhibit the tonic contractions. These results are consistent with data presented in this study. Flunixin meglumine has shown its significant inhibitory effect despite species differences.

Hoogmoed et al. (2000) showed in *vitro* effect of flunixin meglumine, carprofen, ketoprofen, and phenylbutazone on horse smooth muscle tissues collected different locations of the colon. All NSAIDs dose-dependently inhibit contractions in colon tissues regardless of location and muscle orientation. The results of this study,

coupled with our findings that flunixin meglumine significantly stopped the contractions in the colon regardless of species difference, suggests that flunixin meglumine can be an effective treatment for the pain related to the large intestine.

In *vitro* investigation of COX inhibitors, flunixin meglumine, and firocoxib was performed on equine jejunum by Wogatzki et al. (2017). Flunixin meglumine significantly reduced the contractility of experimental tissues, whereas firocoxib achieved no significant effect, leading to a conclusion that difference in results could be possibly due to difference in selectivity and difference in inhibition of COX enzymes via NSAIDs.

In isolated colon samples of ponies, flunixin meglumine induced an increase in myoelectric activity in the right ventral colon and accelerated caecal emptying rate (Lester et al., 1998) which are opposite to data present in this study where flunixin meglumine decreased the contractions in colon tissues.

In *vivo* investigations on ponies by Adams, et al. (1984), flunixin meglumine showed no significant effect on the motility of the jejunum or pelvic flexure. However, the parts of GIT were different, the findings of this study contradict with the presented study results.

Data of the present study on abomasum tissue is inconsistent with the study of Mendel et al. (2018) who reported that flunixin meglumine caused the dose-dependent increase in contractility of abomasum and duodenum preparations of cows with the only difference of magnitude of their effect between gut segments.

In *vitro* study conducted by El-Rwegi et al. (2015) to clarify the pharmacological effect of piroxicam and meloxicam on intestinal smooth muscle contractility of rabbit. Interpretation of results shows that each drug induces dose-dependent relaxation in different intestinal segments of rabbit with minimal and maximal effects of more potency by piroxicam than meloxicam. Moreover, the inhibitory action of drugs attributed to inhibiting endogenous PG synthesis through their established action on COX. Findings of this study coupled with the presented study results, showing the inhibitory action of meloxicam on smooth muscles despite of specie difference.

Meloxicam selectivity towards COX-2 inhibition as compared to flunixin meglumine was studied by Little et al. (2007) in ischemia-injured equine jejunum. IV injection of both drugs comparably improved the postoperative pain in the jejunum. COX-1 derived PGs are required for the recovery of intestinal barrier function, and meloxicam seems to inhibit the deleterious effects related to COX-2 derived PGs. Results led to the suggestion that meloxicam can be alternative to flunixin meglumine in the treatment of pain related to colic in horses. On the contrary, in a comparative randomised prospective study between flunixin meglumine and meloxicam in horses postoperatively, flunixin meglumine showed a more significant effect in reducing pain than meloxicam, but there was no difference in outcome between the two groups (Naylor et al., 2014). However, this study was statistically inconclusive, and meloxicam was used at twice the recommended dose, possibly effecting the level of selectivity of meloxicam. A study by Erol, et al. (2020) evaluated the comparative effects of ketoprofen and flunixin meglumine in postoperative pain in cows undergo an abomasal displacement surgery. Flunixin meglumine was found to be more effective than ketoprofen in postoperative pain control. Results of in *vivo* studies showing the effectiveness of flunixin meglumine and meloxicam is compatible with our in *vitro* findings. We showed that both drugs are effective in reducing the pain related to the GIT at their appropriate doses.

Atropine is well known for its inhibitory effect on motility. The drug reduced the contractions by antagonising the effect of ACh in the jejunal smooth muscle tissue of horses. (Menozzi et al., 2017). Similarly, atropine showed a greater and last longer effect in decreasing motility of the distal portion of the jejunum and pelvic flexure of ponies (Adams et al., 1984). Also, atropine blocked the contractions in the longitudinal muscle of the guinea pig isolated ileum (Holzer et al., 1997). In quiescent abomasum isolated segments of sheep, atropine did not abolish the contractions at the concentration of $3.9x10^{-6}$ M while at the same concentration the drug stopped the ACh evoked contractions (Wong and McLeay, 1988). In the present study, atropine was used as positive control drug because of its inhibitory action on GIT motility. The drug showed attribute to its antagonist effect by blocking the CH evoked contractions in the ileum and proximal loop of the colon, which is similar to the results of previously mentioned studies, but the drug failed to inhibit the contractions in the abomasum and centripetal gyri of the colon which shows the

contradiction in the effect of atropine between our and previous studies. Differences in results most likely emphasize species and contractile drugs differences among studies.

In *vitro* investigation of indomethacin, ketoprofen, and nabumetone in the contractions of the smooth muscle of the third compartment of the stomach of llamas, the nonselective NSAIDs induced excitatory response in general, but the indomethacin induced a significant increase in contractile strength of smooth muscles (Hoogmoed et al., 2004) while the drug decreased the contractions in guinea pig ileum (Maggi et al., 1994). Similarly, in rats, indomethacin treatment inhibits the contractile activity in the distal regions of the small intestine (Lichtenberger at al., 2015). In isolated fundus and corpus part of the stomach in dogs, indomethacin inhibited the spontaneous contractions (Milenov and Golenhofen, 1982). A study by Shahbazian et al. (2001) reported that the COX-1 inhibitor SC-560, the COX-2 inhibitor NS-398, and the isoform-non-selective inhibitors flurbiprofen and piroxicam had no major effect on peristalsis, whereas indomethacin and etodolac induced the contractions in the small intestine of guinea pigs. Indomethacin has shown different even opposite results on GIT contractility, which concluded that the effect of the drug depends on the type of species and part of the GIT used in an experiment.

Analgesic effects of acetaminophen, acetylsalicylic acid, and dipyrone were examined in isolated intestinal segments of a guinea pig by Herbert et al. (2005). The results show that acetaminophen dose-dependently decreases the peristaltic movements of intestinal tissues, whereas acetylsalicylic acid and dipyrone lacked such an effect. Inhibitory action of acetaminophen involves transmitters acting through Ca^{2+}-activated potassium channels, endogenous opioidergic pathways, and presumably inhibition of COX- 3. However, it was shown by a study that dipyrone inhibits COX-3 more potently than either COX-1 or COX-2. In sheep, intravenous, oral, and intramuscular injection of lysine-acetylsalicylate at therapeutic doses abolished the electroencephalograms evoked reticular contractions, produce abomasum hypomotility, which was attributed to an α2-adrenergic effect (Honde and Buéno ,1984).

The effect of ibuprofen on postoperative gut motility in dogs was studied by Thayer et al. (1988). Ibuprofen caused a significant increase in motility, and a major effect of the drug was seen on the ileum and proximal colon.

Prostaglandins cause contraction and relaxation of gastrointestinal smooth muscle depending on the type of PGs administered and on the type of muscle layer involved (De Winter et al., 1998). Role of PGs in gut motility by PG inhibitors aspirin and indomethacin in isolated guinea pig ileum and colon were investigated by Bennett, et al. (1976). As findings, aspirin (20-100 μg/ml) and indomethacin (1-4 μg/mL) significantly decreased the peristalsis of gut muscles in both tissues. Results showed that a decrease in peristalsis by NSAIDs occurs due to PG depletion via inhibition of COX, which led to the conclusion that the study results support the hypothesis that PGs contribute to the peristaltic activity. Inhibition of PG and contractions by indomethacin in rat stomach also shows the possibility of involvement of PGs in the preservation of gut motility (Singh, 1980). In dogs, administration of indomethacin increased gastrointestinal motility, which specifies that endogenous PGs are important in the physiological control of intestinal motility (Thor et al., 1985).

Saat (2009) investigated the effect of meloxicam on spontaneous contractility of the myometrium isolated from non-pregnant cow and heifer uterus. Meloxicam at cumulative dose of 1.5 μM significantly reduced the frequency of contractions in myometrial tissue. A study by Das et al. (2012) also reported the inhibitory effects of meloxicam on isolated cattle myometrium tissue. These results are consistent with present study results. Meloxicam showed its significant inhibitory effect on smooth muscles of both GIT and reproductive system. Das et al. (2012) investigated the effects of diclofenac potassium and diclofenac sodium on cattle myometrium. Both NSAIDs dose dependently inhibit the contractions in myometrial tissue.

The inconsistency between the results of the present study and those of other studies is possibly due to difference of NSAIDs used, drug concentrations, different environmental conditions, methods and as well as different experimental animals that have been used.

5. CONCLUSION

Flunixin meglumine and meloxicam are nonsteroidal anti-inflammatory drugs with analgesic, anti-inflammatory property. Results of this study showed that flunixin meglumine showed higher potency by inhibiting the smooth muscle contractions at lower doses than meloxicam. However, despite of low potency of the meloxicam, it is associated with less GI side effects than flunixin meglumine because of its selectivity towards COX-2. So, outcome of this study supports the effective and safe use of both flunixin meglumine and meloxicam in cattle as painkiller, depends on the availability and cost of the respective drug.

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