Kafkas Univ Vet Fak Derg
19 (3): 449-454, 2013
DOI: 10.9775/kvfd.2012.8028

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Tocolytic Effects of Diclofenac Potassium and Diclofenac Sodium on Cattle Myometrium Pre-Incubated with PCB-153 [1]

Yavuz Kursad DAS * Abdurrahman AKSOY * Oguzhan YAVUZ *
Dilek GUVENC * Enes ATMACA *

[1] *This study was presented as a poster presentation at the 12th International Congress of the European Association for Veterinary Pharmacology and Toxicology (EAVPT) from 08 to 12 July, 2012 in Noordwijkerhout, the Netherlands*

* Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ondokuz Mayis University, TR-55139 Samsun - TURKEY

Makale Kodu (Article Code): KVFD-2012-8028

Summary

In this study, the tocolytic effects of the potassium and sodium salts of diclofenac, a non-steroidal anti-inflammatory drug (NSAID), on cattle myometria were investigated in the presence of an estrogenic polychlorinated biphenyl congener 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153). The uterine samples were obtained from newly slaughtered, non-pregnant cattle more than two years old and in the anoestrus stage. Eight groups were constituted for testing (each group contained 8 strips from two animals, with 4 from each); there were four control groups (diclofenac potassium 24 h and 48 h; diclofenac sodium 24 h and 48 h) and four groups for PCB-153 (diclofenac potassium 24 h and 48 h; diclofenac sodium 24 h and 48 h). The control groups were incubated in physiological salt solution (PSS) for 24 or 48 hours and the PCB-153 groups were incubated in PSS which contained 100 ng/mL of PCB-153, for either 24 h or 48 h. After the incubation period, the strips were hung and incubated in the tissue bath system until spontaneous contractions occurred. Oxytocin (OT) was administered at 0.5 nM to the strips in all groups for the stimulation of spontaneous contractions. Diclofenac potassium and diclofenac sodium were then administered cumulatively in the range of $1x10^{-7}$ to $7x10^{-4}$ M to the strips of all groups to the maximum inhibitory effect was observed. After each application, isometric uterine contractions were recorded for 20 min. Mean peak amplitude (P_{MAX}), frequency (beats per minutes, BPM) and area under the curve (AUC) values of the myometrial contractions, which are criteria for determining drug effects, were calculated from the curve and evaluated statistically. The inhibitory concentration 50 (IC_{50}) values were calculated for the BPM, P_{MAX} and AUC values of the myometrial contractions. Finally, the tocolytic effects of diclofenac potassium and diclofenac sodium on the contractions of cattle uterine strips pre-incubated with PCB-153, were determined.

Keywords: *Diclofenac potassium, Diclofenac sodium, PCB-153, Cattle, Tocolysis*

PCB-153 İle İnkübe Edilen Sığır Miyometriumu Üzerine Diklofenak Potasyum ve Diklofenak Sodyumun Tokolitik Etkisi

Özet

Bu çalışmada, steroid yapıda olmayan ağrı kesici ve yangı giderici ilaçlardan (non steroidal anti-inflammatory drugs, NSAIDs) diklofenakın potasyum ve sodyum tuzlarının poliklorlu bifenil (PCB)'lerden östrojenik etkili 2,2',4,4',5,5'-hekzaklorobifenil (PCB-153) varlığında sığır uterus düz kaslarını gevşetici etkisi araştırılmıştır. Uterus örnekleri mezbahada iki yaşın üzerinde, yeni kesilen, anöstrüs evresinde, gebe olmayan ineklerden sağlanmıştır. İlaçların etkisini ortaya koymak için, dört kontrol (diklofenak potasyum 24 ve 48 saat, diklofenak sodyum 24 ve 48 saat) ve dört PCB-153 (diklofenak potasyum 24 ve 48 saat, diklofenak sodyum 24 ve 48 saat) olmak üzere sekiz ayrı grup oluşturulmuştur. Her bir grup iki farklı hayvandan dörder olmak üzere toplam sekiz miyometrium şeridi içermektedir. Kontrol grupları ayrı ayrı 24 ve 48 saat süre ile fizyolojik tuz solüsyonu (physiological salt saline, PSS) içinde, PCB-153 grupları ise aynı sürelerle 100 ng/mL PCB-153 içeren PSS içinde bekletilmiştir. Bu süreler sonrasında miyometrium şeritleri izole doku banyosu sistemine asılarak spontan kasılmalar oluşuncaya kadar beklenmiştir. Tüm gruplara spontan kasılmaları teşvik etmek amacı ile 0.5 nM yoğunluğunda oksitosin (OT) uygulanmıştır. Daha sonra diklofenak potasyum ve diklofenak sodyum kendileri için oluşturulan kontrol ve PCB-153 gruplarına $1x10^{-7}$ ve $7x10^{-4}$ M arası yoğunluklarda en yüksek inhibitör etki görülünceye kadar kümülatif olarak uygulanmıştır. Her uygulama sonrası uterus kasılmaları 20 dk süre ile kaydedilmiştir. Elde edilen grafiklerden ilaç etkisinin ölçütleri olan ortalama pik yükseklikleri (P_{MAX}), frekansları (dakikadaki pik sayısı, beats per minute-BPM) ve eğrinin altında kalan alan (EAA) değerleri hesaplanmış ve istatistiksel olarak değerlendirilmiştir. Ayrıca miyometrium kasılmalarının BPM, P_{MAX} ve EAA değerleri için inhibitör yoğunluk 50 (IC_{50}) değerleri hesaplanmıştır. Çalışma sonunda diklofenak potasyum ve diklofenak sodyumun önceden PCB-153 ile inkübe edilen sığır miyometrium şeritleri üzerine tokolitik etkisi ortaya konmuştur.

Anahtar sözcükler: *Diklofenak potasyum, Diklofenak sodyum, PCB-153, Sığır, Tokoliz*

İletişim (Correspondence)
+90 362 3121919/2830
ykdas@omu.edu.tr

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of chlorinated organic compounds which was widely used, particularly in the manufacturing of electrical equipment, because of their favourable dielectric properties and chemical stability [1]. Although many countries have banned or restricted the use of PCBs, their distribution in nature is still ubiquitous. PCBs are bioaccumulated in lipid tissue and biomagnified along food chains. A variety of the 209 PCB congeners are, to different extents, detectable in environmental sampling and in human and animal tissues [2]. These compounds may be responsible for shortening the duration of pregnancy in women. Moreover, a mixture of PCBs (Aroclor 1254) was found to induce abortion in monkeys [3]. PCBs are also suspected of affecting cell function via the estradiol receptor (ER), as agonists or antagonists, and via the aryl-hydrocarbon receptor (AhR). It is well-known that PCB 153, and to some extend PCB 77, have estrogen - like properties. Although some PCBs have a lower affinity for the ER than estradiol, they can stimulate OT secretion from granulosa cells at a higher level than estradiol [4].

OT, a non-peptide hormone, produces uterine and mammary gland contractions. Both tissues contain OT receptors (OTRs). The number of these receptors is increased by estrogen and decreased by progesterone [5]. OT is secreted from granulosa cells in cattle is estradiol-dependent and is involved in growth, maturation and ovulation of follicles [6]. Several studies in ewes have shown that estradiol administration *in vivo* can stimulate endometrial OTR expression [7]. Thus, the concomitant rise in estrogen and fall in progesterone levels that occur before parturition can cause increased sensitivity to OT and result in labour. The drug is used clinically for the induction of labour and to stimulate lactation [5]. In the first days of the estrus cycle, OT is involved in the establishment of the corpus luteum and then OT of luteal origin amplifies luteolysis at the end of the cycle. Hence, disruptions to the synthesis or secretion of OT can impair the estrus cycle and reduce fertility of cattle [6].

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is released from the uterus in large amounts during parturition, and this is commonly assumed to initiate labour contractions in pregnant cows [8]. PGs are well-known uterine contraction inducer by enhancing myometrial gap junction and increasing intracellular calcium concentration [9]. The increase in PG during labour causes increased uterine contractility and inhibitors of PG synthesis delay the onset of labour [10]. OT is a much more potent myometrial stimulant than $PGF_{2\alpha}$ and causes contractions in picomolar concentrations rather than at nanomolar levels. Moreover, the release of PGs from intact tissues *in vivo* is rarely spontaneous and usually occurs in response to some kind of stimulus [8]. The force of myometrial contractions is increased by OT and $PGF_{2\alpha}$. The ability of OT to stimulate contractions of the myometrium rises around ovulation because of an estradiol-dependent increase in the number of OTRs [11].

In obstetrics and gynecology, NSAID's have long been used to control acute and chronic postoperative pain, menstrual pain, pain related to medical abortions, menorrhagia, intrauterine device, assist in fertility treatment, and administered as tocolytics in preterm labor [10]. But their adverse effects, including premature closure of the ductus arteriosus and reduction in renal perfusion that result in decreased amniotic fluid volume, have limited their use in preterm labour [12]. Diclofenac, one of the NSAIDs, is used for pain control in major surgery. The exact mechanism of action is not entirely understood, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic and analgesic activity is inhibition of PG synthesis by inhibition of cyclooxygenase (COX) activity [13] like the other NSAIDs. COX is divided into three subgroups: COX-1, COX-2, and COX-3. COX-1 is constitutively expressed in many mammalian tissues. On the other hand, COX-2 is induced in response to various stimuli, such as cytokines, bacterial lipopolysaccharide (LPS) and growth factors. COX-3 is a splicing variant derived from the COX-1 gene. This protein is expressed in the central nervous system and is involved in the sensitive neuronal pathway [14]. Diclofenac has a low to moderate ability to block COX-2 and is therefore reported to cause a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin [13]. Assessment of inhibitory concentration 50 (IC_{50}) values for COX-1 and COX-2 and the calculation of COX-1/COX-2 IC_{50} ratios for different COX inhibitors show that their biochemical selectivity is a continuous variable. Thus, it is quite difficult to establish COX-1 and COX-2 IC_{50} values that separate non-selective from selective NSAIDs. However, for a cluster of compounds including the profens (ibuprofen, ketoprofen, flurbiprofen) and naproxen, it is apt to classify them as non-selective NSAIDs for their inhibitory effects on both COX-1 and COX-2 activity. The cluster of COX-2 inhibitors should also include the traditional NSAIDs, meloxicam, nimesulide and diclofenac (which are from 18 - to 29 - fold more potent towards COX-2 *in vitro*), and coxibs [15].

When estrogenic activity increases, the risk of preterm labour increases in pregnant animals. PCB-153 is an estrogenic compound. Therefore, the aim of this study was the determination of the tocolytic effect of diclofenac potassium and diclofenac sodium on cattle myometrium pre-incubated with PCB-153 in the isolated tissue bath system.

MATERIAL and METHODS

In this study, the methods described by Çelik et al.[16] and Wrobel et al.[3] were employed. Myometrial strips were obtained from newly slaughtered, non-pregnant cattle more than two years old and in the anoestrus stage at the time of sampling. Extra vascularisation, thickening and caruncles were not observed in the uterine endometriums. Also there was no asymmetry between two uterine horns,

macroscopically. The uterine samples were immediately brought to the laboratory in physiological salt solution (PSS) at 4°C. The PSS for transportation and incubation of the strips in the tissue bath was constituted as follows: 116 mM NaCl, 4.6 mM KCl, 1.16 mM NaH_2PO_4 x $2H_2O$, 1.16 mM $MgSO_4$ x $7H_2O$, 21.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$ x $2H_2O$ and 11.6 mM dextrose (Merck, Germany). Longitudinal myometrial strips (1 cm x 0.2 cm x 0.2 cm) were prepared from the curvature major of the uterine horns. The eight test groups, each containing 8 strips as 4 strips from each of two animals (a Brown Swiss and a Holstein), were constituted as four control groups (diclofenac potassium 24 h and 48 h; diclofenac sodium 24 h and 48 h) and four groups for PCB-153 (diclofenac potassium 24 h and 48 h; diclofenac sodium 24 h and 48 h). The control groups were incubated in PSS and the PCB-153 groups were incubated in PSS which contained 100 ng/mL of PCB-153 [3] (Dr.Ehrenstorfer, Germany) at 4°C for either 24 h or 48 h. After the incubation period, the strips were hung on force transducers (Commat, Turkey) and 2 g of basal tension was applied to the strips in 10 mL tissue baths (95% O_2 + 5% CO_2, pH 7.4, 38°C). They were incubated in tissue baths until spontaneous contractions occurred. OT (Sigma, USA) was administered at 0.5 nM to the strips in all groups to stimulate spontaneous contractions. After that, diclofenac potassium and diclofenac sodium (Novartis, Turkey) were administered cumulatively at $1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$, $1x10^{-4}$, $2x10^{-4}$, $3x10^{-4}$, $4x10^{-4}$, $5x10^{-4}$, $6x10^{-4}$ and $7x10^{-4}$ M to the strips of both the control and PCB-153 groups until the maximum inhibitory effect was observed. After each application, isometric uterine contractions were recorded with a data acquisition system (Biopac, USA) for 20 min.

P_{MAX}, BPM and AUC values of the myometrial contractions, which are criteria for determining drug effects, were calculated from the curve. Factorial Analysis of Variances (ANOVA), followed by the Tukey's Post Hoc Test, were used for statistical evaluation. The effects of PCB-153 on AUC, P_{MAX} and BPM values for spontaneous cattle uterine contractions before and after OT application were determined with the Independent Samples T Test. Differences were considered significant when P values were less than 0.05. In addition, the IC_{50} values of diclofenac potassium and diclofenac sodium were calculated for the BPM, P_{MAX} and AUC values of the myometrial contractions with the GraphPad Prism (5.0) program.

RESULTS

The effects of diclofenac potassium and diclofenac sodium on the isometric contractions of cattle myometrium pre-incubated with PCB-153 for a 24 h period and stimulated with OT are shown in *Fig. 1* and *2* as examples.

The statistical analyses of AUC, P_{MAX} and BPM showed that the time x drug relationship for both diclofenac potassium and diclofenac sodium was significant ($P<0.05$), while the dose x time, dose x group (control and PCB-153), time x group and dose x time x group relationships were not found significant ($P>0.05$).

The effects of diclofenac potassium and diclofenac sodium on the AUC values of the isometric cattle myometrial contractions are shown in *Fig. 3*. The mean AUC value of the PCB-153 48 h group was found significantly

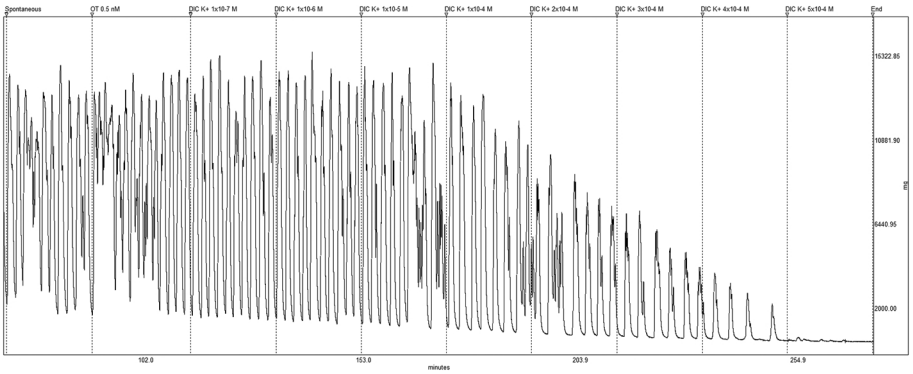


Fig 1. The effects of diclofenac potassium on the isometric contractions of cattle myometrium pre-incubated with PCB-153 for a 24 h period and stimulated with OT

Şekil 1. 24 saat süresince PCB-153 ile inkübe edilen ve OT ile uyarılan izometrik sığır miyometrium kasılmaları üzerine diklofenak potasyumun etkisi

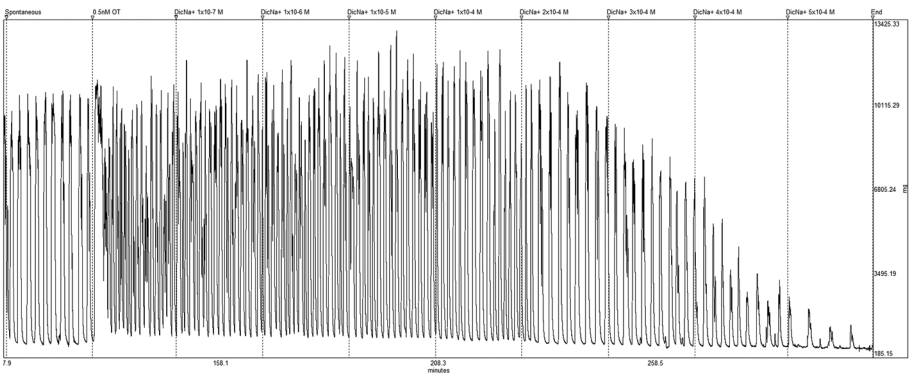


Fig 2. The effects of diclofenac sodium on the isometric contractions of cattle myometrium pre-incubated with PCB-153 for a 24 h period and stimulated with OT

Şekil 2. 24 saat süresince PCB-153 ile inkübe edilen ve OT ile uyarılan izometrik sığır miyometrium kasılmaları üzerine diklofenak sodyumun etkisi

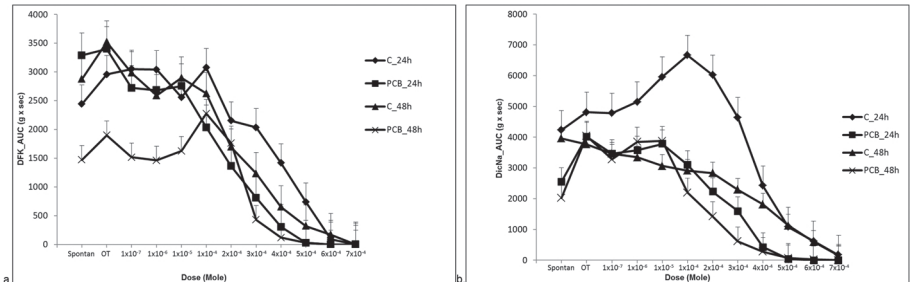
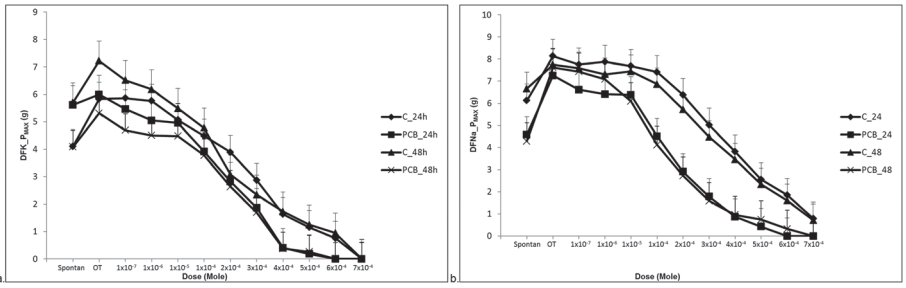


Fig 3. The effects of (a) diclofenac potassium and (b) diclofenac sodium on the AUC values of isometric cattle myometrial contractions

Şekil 3. Diklofenak potasyum (a) ve diklofenak sodyumun (b) izometrik sığır miyometrium kasılmalarının EAA değerine etkisi

Fig 4. The effects of (a) diclofenac potassium and (b) diclofenac sodium on the P_{MAX} values of isometric cattle myometrial contractions

Şekil 4. Diklofenak potasyum (a) ve diklofenak sodyumun (b) izometrik sığır miyometrium kasılmalarının P_{MAX} değerine etkisi



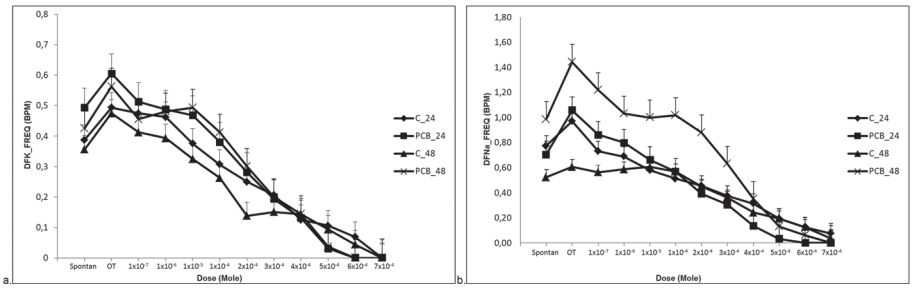


Fig 5. The effects of (a) diclofenac potassium and (b) diclofenac sodium on the BPM values of isometric cattle myometrial contractions

Şekil 5. Diklofenak potasyum (a) ve diklofenak sodyumun (b) izometrik sığır miyometrium kasılmalarının BPM değerine etkisi

Table 1. *The IC_{50} values with 95% confidence limit for mean (CLM) of diclofenac potassium and diclofenac sodium for BPM, P_{MAX} and AUC for cattle uterine contractions*

Tablo 1. *Diklofenak potasyum ve diklofenak sodyumun sığır uterus kasılmalarının BPM, P_{MAX} ve EAA'ları üzerine % 95 güven aralığı ile birlikte IC_{50} değerleri*

Values	Diclofenac Potassium				Diclofenac Sodium			
	Control 24 h	PCB-153 24 h	Control 48 h	PCB-153 48 h	Control 24 h	PCB-153 24 h	Control 48 h	PCB-153 48 h
P_{MAX}	$1.75x10^{-3}$	$5.32x10^{-4}$	$5.19x10^{-4}$	$2.51x10^{-4}$	$5.38x10^{-4}$	$2.25x10^{-4}$	$6.31x10^{-4}$	$2.02x10^{-4}$
95% CLM	$0.46\text{-}6.68x10^{-3}$	$2.28\text{-}12.41x10^{-4}$	$2.50\text{-}10.77x10^{-4}$	$1.95\text{-}3.21x10^{-4}$	$4.00\text{-}7.25x10^{-4}$	$1.81\text{-}2.80x10^{-4}$	$4.00\text{-}9.94x10^{-4}$	$1.32\text{-}3.08x10^{-4}$
BPM	$6.62x10^{-4}$	$7.68x10^{-4}$	$3.05x10^{-4}$	$1.26x10^{-3}$	$1.78x10^{-3}$	$5.25x10^{-4}$	$4.76x10^{-4}$	$3.50x10^{-4}$
95% CLM	$2.11\text{-}20.75x10^{-4}$	$4.10\text{-}14.38x10^{-4}$	$0.10\text{-}9.32x10^{-4}$	$0.42\text{-}3.73x10^{-4}$	$0.23\text{-}13.97x10^{-3}$	$1.99\text{-}13.88x10^{-4}$	$2.34\text{-}9.67x10^{-4}$	$2.61\text{-}4.70x10^{-4}$
AUC	$5.32x10^{-4}$	$2.29x10^{-4}$	$2.86x10^{-4}$	$2.80x10^{-4}$	$3.77x10^{-4}$	$2.75x10^{-4}$	$1.19x10^{-3}$	$1.57x10^{-4}$
95% CLM	$1.46\text{-}19.36x10^{-4}$	$1.76\text{-}2.98x10^{-4}$	$2.04x3.99x10^{-4}$	$0.72\text{-}10.88x10^{-4}$	$3.11\text{-}4.48x10^{-4}$	$2.04\text{-}3.72x10^{-4}$	$0.51\text{-}2.77x10^{-3}$	$0.98\text{-}2.51x10^{-4}$

Table 2. *The effect of PCB-153 on AUC, P_{MAX} and BPM values for spontaneous cattle uterine contractions before and after OT application (%)*

Tablo 2. *PCB-153'ün spontan sığır uterus kasılmalarının AUC, P_{MAX} and BPM değerleri üzerine OT uygulama öncesi ve sonrası etkisi (%)*

Values	Control 24 h	PCB-153 24 h	Control 48 h	PCB-153 48 h
	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
AUC	68.54±36.20	37.66±13.08	37.82±19.93 [a]	156.63±44.7 [b]
P_{MAX}	39.24±6.35	32.17±7.68	31.64±8.98 [a]	80.33±21.18 [b]
BPM	34.83±10.87	40.77±8.82	26.83±6.81	43.92±10.57

[a,b] Means within the same row with different letters are significantly different (P<0.05)

different from the other 3 groups (P<0.05) and there was no difference between the other groups for diclofenac potassium (P>0.05). In the 24 h control group, diclofenac sodium was found significantly different from the other groups (P<0.05). For the various doses, there was no difference between the AUC value for spontaneous

contraction and the dose until $1x10^{-4}$ M (P>0.05). However, a significant tocolytic effect was determined after the $2x10^{-4}$ M dose in both diclofenac sodium and diclofenac potassium applications (P<0.05).

The effects of diclofenac potassium and diclofenac sodium on the P_{MAX} values of isometric cattle myometrial contractions are shown in *Fig. 4*. There was no significant difference between the respective control groups and diclofenac potassium and diclofenac sodium applications (P>0.05). However, the PCB-153 24 h and the PCB-153 48 h groups were significantly different from the control groups (P<0.05) for both diclofenac potassium and diclofenac sodium. A significant difference was also observed between the P_{MAX} values for spontaneous contraction and after OT application (P<0.05). This difference disappeared in the $2x10^{-4}$ and $3x10^{-4}$ M concentrations (P>0.05), and a significant tocolytic effect occurred at the doses higher than $4x10^{-4}$ M concentration (P<0.05).

The effects of diclofenac potassium and diclofenac sodium on the BPM values of isometric cattle myometrial contractions are shown in *Fig. 5*. There was no difference between the control and PCB-153 groups for diclofenac potassium (P>0.05) and between the control groups (P>0.05). However, the PCB-153 24 h and the PCB-153 48 h groups were significantly different from their respective control groups (P<0.05) for diclofenac sodium. In addition, the PCB-153 24 h group was significantly different from the PCB-153 48 h group (P<0.05). For doses, the significant difference between BPM values of spontaneous contraction and after OT application (P<0.05) disappeared between the doses from $1x10^{-7}$ to $1x10^{-4}$ M (P>0.05), and the tocolytic effect was significant at higher molarities than $2x10^{-4}$ M for both diclofenac potassium and sodium (P<0.05). The tocolytic effect was significant from $4x10^{-4}$ to $7x10^{-4}$ M (P<0.05), and it peaked in $6x10^{-4}$ and $7x10^{-4}$ M.

The IC_{50} values of diclofenac potassium and diclofenac sodium for the BPM, P_{MAX} and AUC values of cattle myometrial contractions are shown in *Table 1*. The lowest IC_{50} values for P_{MAX} and AUC were determined in the PCB-153 48 h group of diclofenac sodium and the lowest IC_{50} value of BPM was determined in the 48 h control group of diclofenac potassium. The highest IC_{50} value for AUC was in the 48 h control group of diclofenac sodium. The highest IC_{50} value for P_{MAX} was in the 24 h control group of diclofenac potassium and the highest IC_{50} value of BPM was in the 24 h control group of diclofenac sodium.

The effects of PCB-153 on AUC, P_{MAX} and BPM values for spontaneous cattle uterine contractions before and after OT application were shown in *Table 2*. There was no significant difference between the control 24 h group and the PCB-153 24 h group (P>0.05). However, a significant increase was observed in the AUC and P_{MAX} values of the PCB-153 48 h group when compared with the control 48 h group (P<0.05). There was also an increase in the BPM value of the PCB-153 48 h group when compared with the control 48 h group, but that change was not significant (P>0.05).

DISCUSSION

Wrobel et al.[3] investigated the *in vitro* effects of PCBs on the contractility of bovine myometrium from the periovulatory stage of the estrus cycle. Uterine strips were incubated with Aroclor 1248, a mixture of PCBs, or with one of three PCBs (PCB-77, -126 or -153), all at doses of 10 or 100 ng/mL. Aroclor 1248 increased the force of spontaneous contractions after 24 h but it decreased after 48 h. Pretreatment with the estrogen-like PCB-153 increased the frequency of both spontaneous and OT-induced myometrial contractions. The authors concluded that PCBs may impair both fertilization and blastocyst implantation in cows.

Wrobel et al.[17] reported that PCB-153 did not alter myometrial contractility in an acute manner (90 min) in rats but it increased the force of contractions in cattle after 24, 48 and 72 h. They also reported that an increase of $PGF_{2\alpha}$ secretion is involved in causing the adverse effects of PCBs on myometrial contractions.

Tsai et al.[18] reported that 4-Hydroxy-2',4',6'-Trichlorobiphenyl (4-OH - TCB or 4-OH - PCB-30) increased the contractile responses of mid-gestation uteri to OT by an ER-mediated mechanism. A 20 h exposure to either 4-OH - PCB-30 (0.1, 1 or 10 μM) or estradiol-17β (10 μM) failed to alter the contractile response to cumulative additions 10^{-10} to 10^{-7} M. However 42 h exposure to 1 μM 4-OH - PCB-30 or 10 μM estradiol-17β significantly elevated the contractile response to OT.

Kotwica et al.[19] investigated the influence of PCBs and phytoestrogens *in vitro* on the functioning of the reproductive tract in cows. They reported that PCBs increased OT secretion from granulosa cells but paradoxically each congener decreased FSH-stimulated OT secretion. Likewise, congeners and Aroclor 1248 stimulated OT secretion from luteal cells, although that effect was dependent on the stage of the cycle and type of congener, while the effect of PCBs on LH-stimulated OT secretion was equivocal during the course of the estrus cycle.

Thaina et al.[5] investigated the uterine relaxant effects of *Curcuma aeruginosa* Roxb. rhizome extracts on both non-stimulated, agonist- and KCl-stimulated rat uteri. In the non-stimulated uteri, the two extracts (10 and 400 mg/mL) had no significant effect. In contrast, in the stimulated uteri, the chloroform and methanol extracts exerted concentration-dependent inhibition of the contractions induced by OT, $PGF_{2\alpha}$, acetylcholine and KCl with IC_{50}s of 31.4, 58.59, 56.21 and 29.28 μg/mL; and 57.79, 69.3, 223.8 and 69.19 μg/mL, respectively. They also reported that the IC_{50} of diclofenac for $PGF_{2\alpha}$-induced contractions was 31.36 μg/mL.

Lee et al.[20] studied the therapeutic effects of COX in-

hibitors with different isoform selectivity on LPS-induced preterm birth in mice. Preterm birth occurred in 90% of mice after intra-peritoneal LPS injection and in 20% of mice after phosphate-buffered saline solution injection. Indomethacin and meloxicam, but not diclofenac, significantly decreased the incidence of preterm birth induced by LPS to 33.3% and 33.3%, respectively. They concluded that meloxicam appeared to have no advantage over indomethacin with regard to tocolysis and maternal side effects.

In the current study, the tocolytic effects of diclofenac potassium and diclofenac sodium on the contractions of cattle uterine tissues pre-incubated with PCB-153 were similar to those reported by Thaina et al.[5]. However, the results of the *in vivo* study performed by Lee et al.[20] contradict the results of the present study. The estrogenic effects of PCB-153 on AUC and P_{MAX} values were statistically significant after a 48 h incubation period. The BPM value was also affected but not significantly *(Table 2)*. These results are supported by those of Wrobel et al.[3] and Tsai et al.[18]. Our results are also similar to the results of the study performed by Wrobel et al.[17] in terms of 48 h incubation period, but they contradict to us for 24 h period.

Diclofenac potassium is claimed to dissolve faster and hence absorbed faster, than the sodium salt and is recommended for the treatments that need short onset of action, mainly for its analgesic properties [21]. Although there were differences between IC_{50} values of sodium and potassium salts of diclofenac, a statistical evaluation was not performed *(Table 1)*. Because, some of IC_{50} values were low for potassium salt, while some of IC_{50} values were low for sodium salt and there was no correlation between those data. This result may be due to realisation of the study *in vitro* condition.

Although diclofenac is a member of traditional NSAIDs, its 29 fold COX-2 selectivity is satisfactory. Diclofenac may therefore be a useful tocolytic drug for reducing the risk of preterm labour in cases of exposure to PCB-153 or other estrogenic agents. However, *in vivo* studies should be performed on cattle for confirmation of the present results.

Acknowledgements

The authors thank Dr. Serhan Sevgi of Novartis, Turkey for providing the potassium and sodium salts of diclofenac and Gregory T. Sullivan of OYDEM, Ondokuz Mayis University in Samsun, Turkey for editing the English in this manuscript.

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