Effects of pentagamavunon-0 (PGV-0) as alternative analgesics on orthodontic tooth movement in rats

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ABSTRACT

Introduction: Some analgesic drugs may have adverse effects on bone remodelling and, thus, on orthodontic tooth movement rate (OTM). GV-0 is synthesized by reacting vanillin and cyclopentanone catalyzed in acidic condition, and it has been revealed as a selective COX-2 inhibitor. This study was aimed to investigate the effect of pentagamavunon-0 (PGV-0), one of the curcumin analogues, on OTM. **Methods:** This study was conducted on 50 male Wistar rats (350-450 g) which were randomly divided into five groups (n = 10 each): 1) no treatment group (NT), 2) orthodontic treatment only (ORT), 3) ORT plus 0.4% sodium carboxymethyl cellulose (Na-CMC) analgesic carrier, 4) ORT plus 200 mg/kg BW Paracetamol (PCT) as the positive control, and 5) ORT plus PGV-0 (50 mg/kg BW (PGV-0). **Results:** Drug and day interaction was statistically significant on two-way ANOVA. Post-hoc analyses showed that OTM increased from day 3 to 7 in all orthodontic groups over the same distance (p>0.05). Maximum OTM was found on day 6, which was significantly farther than the distance on day 4. On day 7, OTM was less than on day 6. OTM in all orthodontic groups, including in the PGV-0 group, was higher than in the NT group (p<0.05). No differences was seen in OTM between PGV-0 group and other orthodontic groups (p>0.05). Post-hoc analysis (intra days) revealed that OTM in PGV-0 and other orthodontic treatment groups increased. **Conclusion:** After a single orthodontic force, PGV-0 does not inhibit tooth movement in rats from day 1 to day 7. Therefore, it is possible to develop PGV-0 as an alternative analgesics during orthodontic therapy.

Keywords: Analgesic drug, orthodontics, tooth movement, curcumin.

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INTRODUCTION

Orthodontic pain occurs due to the release of inflammatory mediators, especially prostaglandin, that stimulates the nerve endings around the teeth and their supporting tissues.[1,2]

In bone remodelling, osteoclasts will resorb the bone at the direction of the force and deposit osteoblasts at the tension site, thus enabling the tooth to move.[3,4] The differentiation process of osteoclasts and osteoblasts occurs in a harmonious orchestra of induction mechanisms between periodontal ligament (PDL) cells, bone cells, and physical or chemical stimulus factors such as cytokines and growth factors. Of all mechanisms involved, the arachidonic acid cascade is the most predominant process as it produces prostaglandin, particularly prostaglandin E2 (PGE2).[1,2]

When cell homeostasis is disrupted, the arachidonic cascade will be induced by enzymes, degrading the membrane-bound phospholipids into arachidonic fatty acids. Cyclooxygenase (COX) converts the arachidonic acid into unstable cyclic endoperoxides from which prostaglandins, one of the prostanoids besides prostacyclins and thromboxanes, are derived.[5,6] This COX pathway can make use of Cyclooxygenase-1 (COX-1) for the process in the body, Cyclooxygenase-2 (COX-2) which emerges in inflammatory processes, or Cyclooxygenase-3 (COX-3) as the derivatives of COX-1, which is mostly found in the brain.[7,8]

Non-Steroids Anti Inflammatory Drugs (NSAIDs) are analgesics that are often used to relieve pain by blocking the COX pathway.[9,10] The inhibition of prostaglandin production through the COX-2 pathway is known to cause inhibition of orthodontic tooth movement.[11] Orthodontic pain can occur for 2-4 days after every activation of the orthodontic appliance.[12] The search for analgesics that do not cause adverse effects, such as inhibition of the remodelling process, and can guarantee a safe and repetitive use during orthodontic treatment which takes an average of 2 to 3 years, is essential.

Paracetamol (Figure 1C) works through COX-2 and does not inhibit the tooth movement in vivo[11]; however, it has hepatotoxic side effects.[13] Pentagamavunone-0(2,5-bis-(4'-hydroxy-3'-methoxybenzylidine cyclopentanone) (PGV-0) is one of the curcumin analogs[14] (Figure 1A and 1B)

and it is the active molecule of the curry spice turmeric.[15] PGV-0 is synthesized by reacting vanillin and cyclopentanone catalyzed in acidic condition.[16]
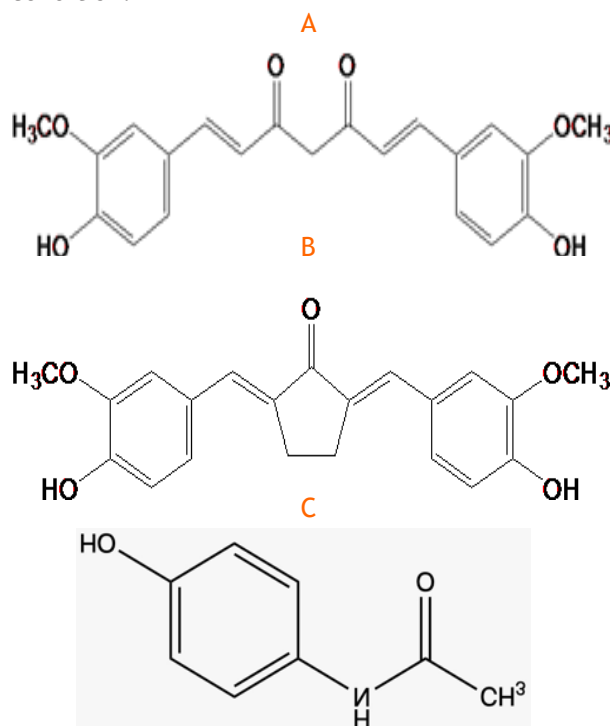

Figure 1.A) Curcumin ((1E,6E)-1-(4-hydroxy-3-methoxyphenyl)-7-(3- methoxy-4-methylphenyl) hepta-1,6-diene-3,5-dione); B) PGV-0 ((2E,5E) -2- [(4-hydroxy-3-methoxyphenyl) methylidene] -5- [(3- methoxy-4-methylphenyl) methylidene] cyclopentan-1- one); C) Paracetamol (N-acetyl-para-aminphenol)

This analog works through COX-2 and is known to have a higher analgesic effect than paracetamol[17] without causing side effects due to its high antioxidant properties.[18] This study was aimed to analyze the effects of PGV-0 on orthodontic tooth movement from day 1 to day 7 after orthodontic force application in rats.

METHODS

This experimental study was conducted on 50 male Wistar rats (350-450 g) and has been approved ethical aspects of experimental research with experimental animals by the Ethical Committee of the Faculty of Dentistry of Gadjah Mada University, Indonesia: 009977/KKEP/FKG-UGM/EC/2017. A metal band was cemented on each incisor of the rats. A force of 35 cN was applied using a 0.012-inch stainless steel coil spring (Ortho Prime Inc. USA; AW 85021201; orthoshape SS 0.012") to separate the two upper incisors.

The animals were randomly grouped into 5 groups (n = 10 each): 1) without treatment group (NT), 2) orthodontics only (ORT), 3) orthodontics with 0.4% sodium carboxymethyl cellulose (Na-CMC) analgesic carrier, 4) orthodontics with 200mg/kg BW Paracetamol (PCT) and 5) orthodontics with (50mg/kg BW PGV-0 (PGV-0).

All drugs were given orally one hour before the start of the OTM and every 12 hours for seven days. OTM was measured daily at the same time using digital sliding callipers (Mitutoyo, Japan). Every measurement was done twice with a 10 minutes interval in three repetitions by an observer. OTM was consistently measured between the incisors at the same point of the uppermost mesial side of the metal band around each incisor. OTM values were calculated by subtracting the distance measured on day 0 from a distance measured at each examination day.

All statistical analyses were performed using SPSS version 22 (Chicago, IL, USA). Means and standard deviations for OTM were calculated in mms. The reliability coefficients between the two measurements taken by one observer were calculated as Pearson's correlation coefficients. The duplicate measurement error (DME) was calculated as the SD of the difference between the two observations divided by $\sqrt{2}$. Paired sample t-tests were applied to identify the systematic differences between the first and second measurements.

The effect of drugs and day of the examination, as well as the interaction of the two variables were analyzed using two-way ANOVA. The comparisons of OTM between the two drugs and on each examination day were analyzed using one-way ANOVA. The significance level = $p < 0.05$.

RESULTS

Error of the methods
Pearson's correlation coefficients for the intra-observer reliability ranged from 0.091 to 0.993, with the lowest for the NT group (Table 1). The duplicate measurement error (DME) was small and varied from 0.045 to 0.298 mm. The mean differences between the first and second measurement were very small. There was only one significant difference between the first and second measurement (PGV-0 on day 5).

Orthodontic tooth movement
During the experiment, one rat died in the Na-CMC, PCT, and PGV-0 groups, respectively. Table

Table 1. Intra-observer performance for measurement of interincisor distance

		Reliabilty	DME	Mean diff.	P-value
Day 0	NT	0.266	0.156	-0.066	0.366
	ORT	0.880	0.115	-0.077	0.165
	Na-CMC	0.588	0.152	-0.006	0.934
	PCT	0.591	0.201	-0.060	0.520
	PGV-0	0.604	0.150	-0.083	0.246
Day 1	NT	0.491	0.153	0.030	0.670
	ORT	0.856	0.155	-0.018	0.801
	Na-CMC	0.922	0.111	0.022	0.663
	PCT	0.963	0.085	-0.061	0.140
	PGV-0	0.873	0.137	-0.008	0.894
Day 2	NT	0.601	0.131	0.086	0.177
	ORT	0.868	0.168	-0.092	0.251
	Na-CMC	0.953	0.119	-0.064	0.286
	PCT	0.957	0.096	-0.034	0.448
	PGV-0	0.903	0.155	-0.046	0.547
Day 3	NT	0.259	0.121	-0.051	0.371
	ORT	0.975	0.099	-0.030	0.516
	Na-CMC	0.993	0.097	-0.062	0.210
	PCT	0.932	0.156	-0.062	0.400
	PGV-0	0.964	0.085	0.022	0.590
Day 4	NT	0.232	0.170	-0.091	0.263
	ORT	0.984	0.045	-0.013	0.543
	Na-CMC	0.977	0.113	0.023	0.674
	PCT	0.576	0.263	0.103	0.406
	PGV-0	0.844	0.163	-0.074	0.361
Day 5	NT	0.735	0.071	0.026	0.425
	ORT	0.886	0.172	0.139	0.104
	Na-CMC	0.851	0.223	0.022	0.838
	PCT	0.571	0.248	-0.032	0.782
	PGV-0	0.762	0.163	-0.213	**0.024**
Day 6	NT	0.091	0.094	-0.090	0.061
	ORT	0.651	0.179	-0.099	0.247
	Na-CMC	0.947	0.076	-0.065	0.108
	PCT	0.699	0.148	-0.070	0.343
	PGV-0	0.522	0.202	0.079	0.429
Day 7	NT	0.031	0.174	0.062	0.444
	ORT	0.762	0.216	0.001	0.996
	Na-CMC	0.975	0.061	0.043	0.175
	PCT	0.203	0.298	-0.003	0.983
	PGV-0	0.900	0.097	-0.103	0.055

NT: no treatment; ORT: Orthodontics only; Na-CMC: orthodontics plus drug carrier group; PCT: orthodontics plus Paracetamol; PGV-0: orthodontics plus PGV-0.

Table 2. OTM from day 1 to day 7 after orthodontic appliance installation

	NT±SD			ORT±SD			Na-CMC±SD			PCT±SD			PGV-0±SD		
Day 1	0.08	±	0.15	0.32	±	0.25	0.18	±	0.25	0.12	±	0.43	0.14	±	0.28
Day 2	-0.01	±	0.20	0.27	±	0.41	0.28	±	0.35	0.37	±	0.32	0.45	±	0.31
Day 3	0.12	±	0.13	0.62	±	0.52	0.72	±	0.48	0.54	±	0.44	0.48	±	0.31
Day 4	0.39	±	0.19	0.34	±	0.44	0.46	±	0.35	0.37	±	0.40	0.67	±	0.36
Day 5	0.23	±	0.19	0.62	±	0.33	0.58	±	0.52	0.59	±	0.25	0.72	±	0.28
Day 6	0.08	±	0.14	0.7	±	0.47	0.72	±	0.36	0.72	±	0.27	0.86	±	0.28
Day 7	0.00	±	0.14	0.55	±	0.63	0.51	±	0.24	0.72	±	0.40	0.73	±	0.37

Notes: Mean ± Standard Deviation are given in mm. **OTM values were calculated by subtracting the distance measured at day 0 from the distance at the specific examination day.** Values are presented as mean ± standard deviation. NT: no treatment; ORT: Orthodontics only; Na-CMC: orthodontics plus drug carrier group; PCT: orthodontics plus Paracetamol; PGV-0: orthodontics plus PGV-0.

2 presents the mean and SD of OTM during the experimental period, starting from day 1 to day 7. All data were normally and homogeneously distributed ($p > 0.05$).

After seven days of force application, the OTM varied between 0.55 (SD 0.63) and 0.73 (SD 0.37) mm in the experimental groups, while virtually, no movement was seen in the NT group.

Figure 2 depicts the OTM from day 1 to day 7 in the experimental groups and in the control group. OTM distance in the PGV-0 group increased, as also seen in other experimental groups. Statistical analyses showed no significant differences for the OTM between PGV-0 and other orthodontic groups. OTM between some of the measurement days was significantly different, as presented in Table 3, however, there was no significant difference seen for the OTM between all orthodontic groups on a certain day, which is presented in Table 4.
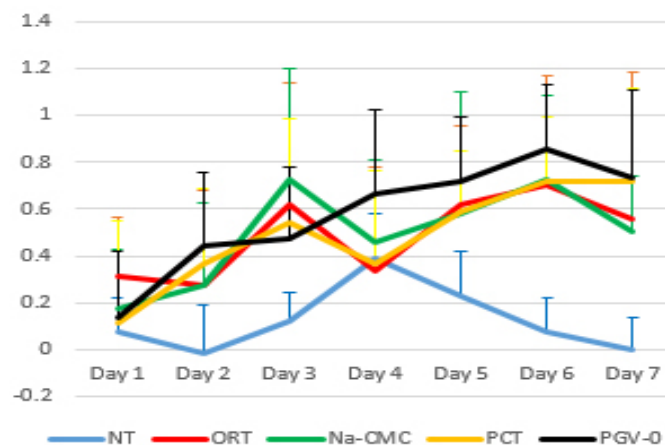

Figure 2. OTM (in mm) from day 1 to day 7 after a single orthodontic force. NT: no treatment; ORT: Orthodontics only; Na-CMC: orthodontics plus drug carrier group; PCT: orthodontics plus Paracetamol; PGV-0: orthodontics plus PGV-0; Statistical analysis showed that OTM was the same for all orthodontic groups from day 1 to day 7. PGV-0 and control groups were not significantly different inter- or intra-days. There was an interaction between the day of examination and drugs factors ($p < 0.05$). Post-hoc comparison of one- and two-way ANOVA can be seen in Table 3 and 4.

Two-way ANOVA showed a significant difference between days of examination and between drug groups, as well as a significant interaction between both factors. The following post-hoc comparison between days revealed that OTM started to increase significantly on day 3. The OTM distance on day 2 was significantly different from the OTM distance on day 3, 4, 5, 6, and 7 with no significant movement from day 3 to day 7. On day 6, the OTM distance was significantly larger than on day 4. Post-hoc comparison between PGV-0 and all other groups showed that the OTM was larger than in the NT group, albeit insignificant ($p > 0.05$).

One-way ANOVA and post-hoc test results showed that the majority of the daily OTM of all orthodontic groups was significantly larger than in the control group except for day 1 and day 4 (p > 0.05). Still, no significant differences was seen between the orthodontic groups (Table 3 and 4).

Table 3. Two-way ANOVA and post hoc test on the effect of PGV-0 on OTM from day 1 to day 7.

Two-way ANOVA	P-value	Post hoc (LSD)
Drugs	< 0.001	ORT*, Na-CMC*, PCT*, PGV-0*
Day	< 0.001	Day 3**, Day 4**, Day 5**, Day 6**, Day 7**
		Day 3***, Day 4***, Day 5***, Day 6***, Day 7***
		Day 6****
Drugs x * Day	0.025	

Notes: OTM values were calculated by subtracting the distance measured at day 0 from the distance at a certain examination day. [a]Tested by two-way analysis of variance, and post-hoc LSD analysis test; NT: no treatment; ORT: Orthodontics only; Na-CMC: orthodontics plus drug carrier group; PCT: orthodontics plus Paracetamol; PGV-0: orthodontics plus PGV-0; *significantly higher than NT (p < 0.05); **significantly higher than day 1 (p < 0.05); ***significantly higher than day 2 (p < 0.05); ****significantly higher than day 4 (p < 0.05)

Table 4. One-way ANOVA and post hoc test on the effect of PGV-0 in day 1 until day 7 on OTM.

	One-way ANOVA (P value)	Post hoc (LSD) (P value)
Day 1	0.535	-
Day 2	0.004	(ORT, NA-CMC, PCT, PGV-0)*
Day 3	0.003	(ORT, NA-CMC, PCT, PGV-0)*
Day 4	0.153	
Day 5	0.003	(ORT, NA-CMC, PCT, PGV-0)*
Day 6	<0.001	(ORT, NA-CMC, PCT, PGV-0)*
Day 7	<0.001	(ORT, NA-CMC, PCT, PGV-0)*

Note: OTM values were calculated by subtracting the distance measured at day 0 from the distance at a certain examination date. Tested by one-way analysis of variance, and post-hoc LSD analysis test; NT: no treatment; ORT: Orthodontics only; Na-CMC: orthodontics plus drug carrier group; PCT: orthodontics plus Paracetamol; PGV-0: orthodontics plus PGV-0; *significantly higher than NT (p < 0.05), no differences between drugs (p > 0.05)

DISCUSSION

Forces created during orthodontic treatments induce the inflammatory mediators, in particular prostaglandins, which in turn stimulates remodeling process, osteoclastic resorption at the pressure side, and osteoblastic apposition on the tension side to move the tooth.[1] However, prostaglandin also stimulates the nerve endings around the teeth and supporting tissues, triggering pain after force application. Non-steroidal Anti-Inflammatory Drugs (NSAIDs) are used to relieve the pain by blocking the cyclooxygenase (COX) pathway of prostaglandin COX-2 synthesis.[11] Inhibition of prostaglandin production, however, may impede orthodontic tooth movement.[11] Paracetamol does not hamper orthodontic tooth movement (OTM) in vivo,[11] but it has a hepatotoxic side effect. An alternative for paracetamol could be PGV-0, which is an active compound of curcumin analog. This analog is known to have a higher analgesic effect than paracetamol without causing adverse effects. This study analyzed the effect of a new curcumin analog, PGV-0, on OTM in comparison to paracetamol as a positive control. PGV-0 has been proven to have a higher analgesic potential than paracetamol, without triggering any side effect such as hepatoxic effects or ulcer digastricus.[15] With regards to orthodontic therapy, the side effects of a certain medication used for pain relief are important because of the long duration of orthodontic therapy. PGV-0 has been known to take its action via COX-2, which is the same as paracetamol.

This study was performed on rats that received a custom-made spring to move the incisors. The spring had not achieved its initial width activation, which could be seen from the activation results at the end of the experiment. The force characteristics of this spring were tested in mice by a previous group.[19] They found that the initial force exerted by the spring at maximum opening (3.5 mm) only showed a minor decrease after constriction to 3.2 mm over a 12-day period. Thus, the force was nearly constant over this period, which fits well with the length of our experimental period.[19]

As presented in Figure 1, the OTM distance generally increased and all orthodontic groups showed the same pattern over a period of 7 days. Two-way ANOVA showed some significant differences in OTM between days. Post-hoc analyses showed no significant differences in OTM between day 1 and day 2. This is in line with the literature stating that the first phase of OTM, that lasts approximately 24 hours to

two days, in which the teeth are moved in their socket only. At the pressure side, the width of the periodontal ligament will be reduced due to the force application and an instantaneous movement of the tooth followed by a slowing down of OTM, probably due to hyalinization of the periodontal ligament.[20-22]

Thereafter, OTM increased significantly from day 3 on. The amount of OTM was significantly different between day 2 and all other days (day 3, 4, 5, 6, and day 7), but OTM from day 3 to day 7 was not significantly different from each other ($p>0.05$). There was a trend of decreasing OTM from day 6 to day 7, but this was not significant. Only the OTM on day 6 was significantly higher ($p<0.05$) in comparison to day 4. Orthodontic tooth movement is characterized by 4 phases: the initial strain phase, the lag phase, accelerated tooth movement phase, and, finally, constant linear tooth movement phase.[23] The lag phase with tooth movement arrest ends after the removal of necrotic tissue, which has been described as occurring between 7 and 21 days in dogs.[24] The necrotic areas in rats are shorter due to its narrower PDL width so it is more prone to force, diminished blood flow, and faster necrotic area induction. This might mean that the OTM in our experiment was probably still in the arrest phase, based on the assumption that the removal of necrotic tissue was in progress.

In an OTM study, prostaglandin-2 (PGE2) levels peaked at 24 h and decreased on day 7 to baseline levels where the orthodontic force decay likely accounted for the decrease in the PGE2 level.[25] The tooth movement on day 7 of our study decreased slightly; however, the value was still higher than on day 2 and the OTM was still the same as on day 3 ($p > 0.05$). Though the drugs were given continuously from one hour before the orthodontic force was applied and twice a day for 7 days, PGE2 seemed still to be produced. All OTM distances had increased significantly until day 7. This means that OTM was not influenced by the type of drug. The significant interaction between day and drugs suggested that the OTM was not only induced by orthodontic force but also by the drugs.

PGV-0 and Paracetamol seemed to stimulate OTM more even though they were not significantly different with OTM in ORT. In studies on COX-2, selective inhibitors NSAIDs has the ability to harm or to postpone bone healing or lessen bone mechanical integrity.[5] In studies on the effect of NSAIDs on bone cells, COX-2 selective inhibitors do not only impair the osteoclast[26], but also the osteoblast.

In a study that was conducted on women aged ≥ 50 year, paracetamol use has been associated with a 56% increase in fracture risk due to its mechanism of action to bone metabolism.[27] Paracetamol has been known to act via COX-2, just like other NSAIDs. In some circumstances, such as in studies on NSAID-induced gastric damage, the inhibition of COX-1 and COX-2 was needed in rats because COX-2 might substitute COX-1 in producing cytoprotective prostaglandins.[7,28]

Prostaglandins have been identified to have a direct effect on osteoclast and osteoblast formations and activities while also maintaining the balance between bone resorption and bone formation.[29,30,31] A study comparing 50% inhibitory concentration (IC_{50}) values in prostaglandin E_2 (PGE_2) production by a marker of monocyte COX-2 activity using bacterial lipopolysaccharide has identified that the IC_{50} for Paracetamol, Indomethacin (an aspirin-like drug) and Diclofenac are 1.6, 1.9 and 29, respectively [32,33] The three types of NSAIDs, i.e. Paracetamol, Aspirin, and Diclofenac, have been known not to inhibit, partly inhibit, and totally inhibit OTM.[11] Paracetamol inhibits both COX-1 and COX-2; therefore, a continued supply of paracetamol twice a day might stimulate OTM more however further studies are still needed.

Currently, no study has been conducted on IC_{50} of COX-1/COX2 of PGV-0 in comparison to paracetamol using bone cells. Nevertheless, the anti-inflammatory and analgesic activities of PGV-0 was found to be higher than those of paracetamol in vivo. The IC_{50} for COX showed that the IC_{50} for PGV-0 is higher than for curcumin. The antioxidant and anticyclo-oxygenase (anti-COX) activity of PGV- 0 is 2 and 7 times higher than curcumin while the anti-inflammatory activity is 5 times higher than curcumin at a same dose (20 mg/kg, per oral).[34]

A post-hoc test of one-way ANOVA intra days showed that on day 3 the OTM in ORT, Na-CMC, PCT. and PGV-0 groups was significantly higher in comparison with the OTM in NT but there were

no differences in the amount of OTM between different experimental groups. On day 4, all groups had the same OTM as the NT group with no differences between groups. Starting from day 5, all orthodontic groups showed an increase in the OTM distance with no differences between all orthodontic groups despite the observation that the maximum movement was achieved in PGV-0 on day 7. Asefi et al. also found that injecting curcumin during OTM to rats does not lead to any inhibitory effect on OTM after 21 days but it inhibit root and bone resorption, osteoclastic recruitment, and angiogenesis significantly.[34] The PGV-0 has been revealed as a selective COX-2 inhibitor[35] that did not only impair the osteoclast but also osteoblast. However, further experiments are still needed.

To summarize, the results of this study showed that PGV-0 and its positive control, paracetamol, did not inhibit OTM despite their action in inhibiting COX-2. There was a significant interaction between variable day and drugs and that the modulation of OTM was not only caused by the drugs but was also influenced by the force decay. PGV-0 could be developed as an analgesic of choice for patients under orthodontic therapy, but further studies are recommended.

CONCLUSION

After a single orthodontic force, PGV-0 does not inhibit tooth movement in rats from day 1 to day 7. Therefore, it is possible to develop PGV-0 as an alternative analgesics during orthodontic therapy.

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