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The Analgesic Effect of a Curcumin Analogue 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadien-3-on (Gamavuton-0) in acute and persistent pain

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ABSTRACT

Gamavuton-0 (GVT-0), a curcumin analogue, has been reported to have antiinflammatory and antioxidant activity, however, its analgesic activity has not yet been investigated. This research purpose is to study the effect of GVT-0 on acute and persistent pain using Modified Hot Plate (MHP) method and Formalin test, respectively, on male Swiss mice. In MHP method, the five groups of animals were pretreated with GVT-0 (10, 20, 40, and 80 mg/kg, orally) and indomethacin (4 mg/kgBB, i.p, for positive control), respectively, and immediately followed by stimulation with the carrageenan in sterile saline with a final volume of 50 µl in the left paw. The animals were then placed in hot plate (51^{o}C). The latency time was determined at 90 min post-challenge. In the Formalin test, the six groups of animals were pretreated with GVT-0 (10, 20, 40 and 80 mg/kg, orally), morphine (5 mg/kgBB, i.p), and indomethacin (4 mg/kgBB, i.p) respectively, 60 min prior to the injection of 1.0% aqueous formalin (20 µl) administered by the intraplantar route into the right hindpaw. The licking time was measured at the first 5 min (initial phase, neurogenic) and 10-30 min after formalin injection (late phase, inflammatory). Result showed that GVT-0 has analgesic effect on acute pain using MHP method with ED_{50} of 27,69 mg/kgBW (p.o). While using Formalin test, GVT-0 showed analgesic activity with ED_{50} of 109,02 mg/kgBW (p.o) in initial phase, and ED_{50} of 13,53 mg/kgBW (p.o) in late phase. These results suggest that GVT-0 is a potential candidate for new antiinflammatory and analgesic agent that can be used for the treatment of different painful condition.

INTRODUCTION

Curcumin (diferuloylmethane), a natural product from the rhizomes of *Curcuma longa* is a yellow colored polyphenolic phytochemical which has been in use for a long time for the treatment of swelling, twisting and wounds. As a pure compound also, it has been found to possess antitumor, anti-cancer and anti-inflammatory activities. Several studies have clearly indicated that curcumin possesses a variety of pharmacological effects such as in anti-inflammatory (Lin *et al*., 2000), anti-carcinogenic (Pan *et al.*, 2000), anti-oxidant (Ramsewak, *et al.*,2000; Somparn, *et al*; 2007), and wound-healing activities (Sindhu *et al.*, 1998).

Gamavuton-0 (GVT-0), with IUPHAC name of 1,5-*bis* (4'-hydroxy-3'-methoxyphenyl)-1,4-pentadien-3-on, is an analogue of curcumin, by modifying the middle site of chemical structure from 1,7-diphenyl-1,6-heptadien-3,5-dion in curcumin to be 1,5-diphenyl-1,4-pentadien-3-on in GVT-0 (Figure 1). Removal of methylene and carbonyl group leads this new compound to become more stable than curcumin without affecting the anti oxidant effect (Sardjiman, 2000). Its anti inflammatory activity was reported to be higher than phenylbutazon and be equal to that of curcumin (Sardjiman, 2000). GVT-0 was also reported to have free radical scavenger activity which was more potent than curcumin (Yuniarti, 2000).

However, there was no report on its analgesic activity so far. Since analgesic activity is often related to the anti inflammatory activity, in the present investigation we study the potential analgesic effect of GVT-0, both in acute and persistent pain in animal models.

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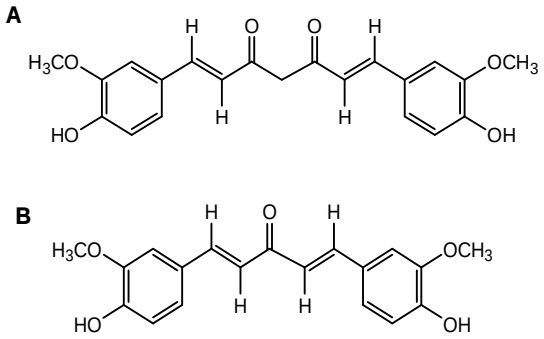

Fig. 1: Comparison between the structure of curcumin (A) and Gamavuton-0 (1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadiene-3-one (B)

MATERIALS AND METHODS

Chemicals

GVT-0 were purchased from Curcumin Research Center Faculty of Pharmacy Gadjah Mada University, Yogyakarta, Indonesia; indomethacin and carragenan was purchased from Sigma Chemical Co. (St. Louis, USA), and morphine was obtained from Kimia Farma tbk., Indonesia. GVT-0, morphine, indomethacin, and carrageenan were dissolved in 0.5% CMC-Na in saline.

Animals

Adult male Swiss albino mice (30 ± 5 g) were obtained from Central Animal House of the Gadjah Mada University. The animals were randomly allocated to treatment groups (six animals per group) in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 27±2°C and relative humidity of 30 to 70%.

A 12:12 light:dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of Faculty of Pharmacy, Gadjah Mada University, Indonesia.

Modified hot-plate test

This method represented a modification of that described by Lavich *et al*. (2005). Forty eight mice were alocated in 8 groups randomly and pretreated with GVT-0 (10, 20, 40, and 80 mg/kg body weight, *p.o*, respectively), indomethacin (4 mg/kg body weight, i.p, for positive control group), and 0,5% CMC-Na for negative control, respectively. One group received no treatment for normal control. Acute pain was then stimulated by injection of 50 µL of carrageenan (500 µg/paw) in sterile saline in the left paw. At 90 min after carrageenan injection, the animals were placed in hot plate surface, which the heat was maintained at the constant temperature of 51°C. The maximum time of placing the animals on the hot plate was 30 s to avoid animal tissue damage. The latency time of the shaking or lifting response was determined manually. The test was carried out at ambient temperature (27± 2ºC) with special care taken to avoid environmental disturbances that might influence the animal's response.

Percent (%) analgesic effect to heat was defined as:

$$\% \text{ Analgesic activity} = \frac{lt - lc}{30 - lc}$$

With: *lc* = mean of latency time of control group, and *lt* = mean of latency time of treated group

Formalin test

This procedure followed that described by Xie *et al*. (2005). Six group of animals were pretreated with GVT-0 (10, 20, 40 mg/kg, p.o), morphine (5 mg/kg body weight, i.p), and indomethacin (4 mg/kg body weight, i.p) respectively, 30 min prior to the injection of 1.0% aqueous formalin (20 µL) administered into the right hindpaw by intraplantar route. The observation was carried out in a 22,5 x 22,5 x 23 cm-sized clear transparent glass chamber. Mirror was positioned below the chamber at a 45º angle for unobstructed observation of the mice paws. The amount of time that the animal spent to lick the injected paw during the first 5 min (initial phase, neurogenic) and 10-30 min after formalin injection (late phase, inflammatory) was measured. Negative control animals received only the vehicle used to dilute GVT-0 (0.5% CMC-Na) and without pretreated received for normal control animal.

Data were calculated according to the following formula:

$$\% \ inhibition \quad licing \quad time = \frac{l.c - l.t}{l.t} X \ 100 \ \%$$

Where *l.c* is mean of total licking time of negative control and *l.t* is mean of total licking time of treatment group.

Statistical analysis

Values are expressed as mean ± SEM. The statistical significance of difference between the means was analysed by one-way non-parametric ANOVA and followed by the Tukey test. $P < 0.05$ was considered significant. Effective dose 50% (ED_{50}) was measured by linear regression used Excel 2008 Software (Microsoft Inc., USA).

Table 1: Antinociceptive profile of GVT-0 on the latency of discomfort assessed by the modified hot plate test in mice and the antinociceptive potency (n = 6).

Treatment	Latency time (detik)	% antinociceptive (%)
Negative control	9,8 ± 0,36	0,00± 1,83
Indomethacin 4 mg/kg i.p	25,91 ± 0,56	79,27 ± 2,86*
GVT-0 40 mg/kgBB	24,82 ± 0,34	77,72 ± 1,72*[a]
GVT-0 20 mg/kgBB	18,95 ± 0,52	38,91 ± 2,61*[a]
GVT-0 10 mg/kgBB	16,07 ± 0,33	29,43 ± 1,67*[a]

Each value represents the mean ± SEM; n = 6; * $p < 0.05$, significantly different from negative control; [a] $p < 0.05$, significantly different compared to the positive control group

RESULTS

Modified hot-plate test

The results of modified hot plate test are shown in Table 1. It shows that pretreatment with GVT-0 (10, 0, and 40 mg/kg) increased the latency response of the animals test. GVT-0 with dose of 10 mg/kg produced 29.43% analgesic activity, while the

maximum antinociceptive actvity was 77.72%, which was obtained at the dose 40 mg/kg, comparable to indomethacin (79.27% at 4 mg/kg, i.p.). After calculation, ED_{50} of GVT-0 in modified hot plate test was found to be 27.69 *mg/kg.*

Formalin induced paw licking in mice

Intraplantar injection of the right hind paw with formalin (5%, 10 µl) evoked a characteristic biphasic response in rats – an initial intense response to pain (0–10 minutes; first phase/neurogenic phase) followed by a slowly rising but longer lasting response (10–60 minutes; second phase/inflammatory phase).

The effect of oral administration of GVT-0 with various doses on the formalin induced paw licking in mice are shown in Table II, as well as the effect of morphine and indomethasin. This result indicate the significant and dose dependent inhibiton both on the neurogenic and inflammatory phase response in the formalin test ($P < 0,05$), as shown in Figure 2. However, the effect of GVT-0 was more strongly shown in inflammatory phase than that in neurogenic phase. ED_{50} of GVT-0 in neurogenic phase was 109,02 mg/kgBB, while in inflammatory phase was 13,53 mg/kg. Morphine (5 mg/kg i.p) as reference compound shows the highest analgesic effect on both phases, while indomethasin seems to be more potent in inflammatory phase than in neurogenic phase.

Tabel. 2: Antinociceptive profile of GVT-0 assessed by the formalin-induced paw licking test in mice (n = 5).

Treatment	Licking time (second)	
	First phase (min 0-5)	Second phase (min 10-30)
Negative control	63,11 ± 1,30	36,06 ± 1,00
Morphine 5 mg/kg	19,22 ± 1,37*	3,59 ± 1,29*
Indomethacin 4 mg/kg	31,80 ± 1,20*	8,89 ± 2,03*
GVT-0 40 mg/kgBB	48,11 ± 0,87*	8,20 ± 1,78*
GVT-0 20 mg/kgBB	50,52 ± 1,60*	15,32 ± 0,84*
GVT-0 10 mg/kgBB	55,56 ± 1,21*	18,71 ± 1,82*

Each value represents the mean ± SEM; n = 5; *p <0.05, significantly different from negative control; [a]p <0.05, significantly different compared to the positive control group.

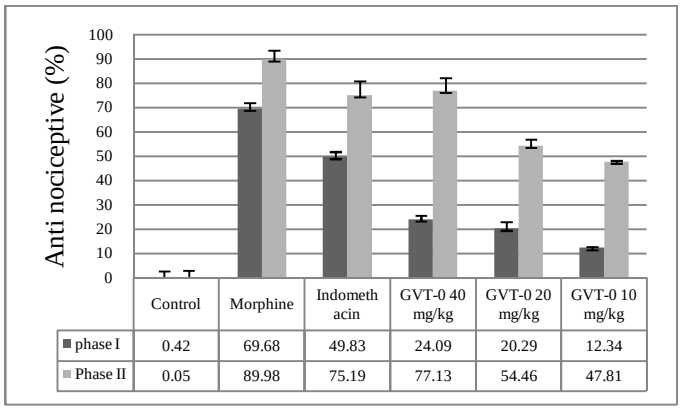

Fig. 2: The analgesic activity of GVT-0 in formalin test. Vehicle and different doses of the GVT-0 were administered 30 min prior to subplantar injection of formalin and the time spent for licking was measured during a 0-5 min (neurogenic phase/phase I) and 20-30 min (inflammatory/ phase II phase) period starting after formalin injection. Morphine (5 mg/kg, i.p.) and indomethacin (4 mg/kg i.p) were used as reference drug. Data are mean ± SEM of 5 animals in each group. All data are different significantly to that of negative control ($P < 0.05$).

DISCUSSION

The results of the present study demonstrates the significant antinociceptive effects of the GVT-0 in both modified hot plate and the formalin test. Modified Hot Plate (MPH) test is a simple and sensitive method for detecting peripheral hyperalgesia and analgesia in rats and mice (Lavich, 2005) and usually used to measure the potential antinociceptive effects of test compounds to an acute thermal stimulus, representing an acute pain. This method use dual activation, i.e. via thermoreceptors (hot plate) and nociceptors (carrageenan), so can cause quicker animal withdrawal from the thermal stimulus compared to the mechanical stimulus. Hyperalgesic changes evoked by carrageenan detected in this MHP test were clearly sensitive to the cyclooxygenase inhibitor indomethacin, which showed 72,27%. inhibition of nociception in this study. The GVT-0, that have antiinflammatory activity (Sardjiman, 2000; Yuniarti (2006), inhibited the nociception in dose-dependent manner. The highest dose of GVT-0 used in this experiment (40 mg/kg) showed comparable anagesic effect to indomethacin (4 mg/kg i.p). It suggest that GVT-0 has analgesic effect against acute pain.

In contrast, the formalin test measures the response to a long-lasting nociceptive stimulus, and may thus bear a closer resemblance to clinical pain. This procedure is used to determine the potential analgesic effects of compounds for states of persistent pain in which tissue damage occurs (Shield, *et al.*,2010). The formalin test is divided into two phases: neurogenic (first phase) and inflammatory (second phase) pain. Central acting analgesics, such as morphine, inhibit both phases. Peripheral acting drugs, such as non-steroid anti-inflammatory and corticosteroids, inhibit only the second phase (Husnkaar and Hole, 1987). The first phase is attributed to direct activation of peripheral nociceptors and sensory afferent fibers by formalin, while the second phase may reflect a combination of low ongoing activity in primary afferents and increased sensitivity of spinal cord neurons.

Our data show that GVT-0 exerts inhibitory effects dominantly during the late phase of the formalin response, and also produced an inhibition of the first phase. Its effect on the first phase was less pronounced, however, significantly different from negative control. This results suggest that this compound may have inhibitory action on direct nociceptor activation, which is usually inhibited by opioid drugs.

The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Husnkaar and Hole, 1987). COX-2 does play an important role in the second phase formalin test **(**Yamamoto and Natsuko, 2002**).** The antinociceptive effect that was evident in this phase of the formalin test may be due to COX inhibitory activity of GVT-0, as reported by Yuniarti (2006).

Curcumin as the parent compound has been reported to have anti inflammatory by inhibiting enzymatic activity of COX-2 (Mukhopadhyay, *et al.*,1982; Pan *et al.*,2000) and also suppress the expression of COX-2 mRNA (Goel, *et al.* 2001; Zhang, *et al.*,1999). Based on the mechanism of curcumin, it might be

hypothesized that GVT-0 also inhibits the expression and enzymatic activity of COX-2. However, the precise mechanism of analgesic effect of GVT-0 still need to be elucidated.

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