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Research Article

SEDATIVE-HYPNOTIC ACTIVITY OF ETHYL- *p*-METHOXYCINNAMATE AND N-(2-HYDROXYETHYL)-*p*-METHOXYCINNAMAMIDE

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ABSTRACT

Ethyl-p-methoxycinnammate (**1**) is a major component of the rhizome of *Kaempferia galanga* that has been found to have a diverse of pharmacological activity such as mosquito repellent and larvicidal, anti-tuberculosis, sedative, anticancer, analgesic and anti-inflammatory and hypo-pigmentary. In this research, we conducted the evaluation of the sedative-hypnotic activity of **1** and its amide derivative, N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) by using diazepam-induced sleep models. Each compound was evaluated in a range of doses of 100, 200 and 400 mg/kg (by oral route) and received diazepam at a dose of 20 mg/kg (Intraperitoneal). Result of assay indicated that compound **1** showed significant decrease onset of sleep at a dose of 100, 200 and 400 mg/kg (p <0.05), and a significant increase in duration of sleep at a dose of 200 and 400 mg/kg (p<0.05), compared to the control group. Compound **2** increase onset sleep at doses of 100, 200 mg/kg (P <0.05), and a significant decrease in duration of sleep at a dose of 100, 200 and 400 mg/kg (P <0.05), compared to control group. Hence, it suggests that compound **1** showed a significant sedative-hypnotic effect (p<0.05), whereas, compound **2** has no significant sedative-hypnotic effect (p>0.05).

Keywords: Ethyl-p-metoxycinnamate (EPMC); *Kaempferia galanga*, N-(2-hydroxyethyl)-p-methoxycinnamamide; sedative-hypnotic activity

INTRODUCTION

Ethyl *p*-methoxycinnamate (EPMC) (**1**) is major component of the rhizome of *Kaempferia galanga* which possessed a broad-spectrum pharmacological and biological activities including sedative[1,2], mosquito repellent and larvicidal[3], anti-tuberculosis[4], anticancer[5], analgesic and anti-inflammatory[6,8] and hypo-pigmentary[9]. EPMC(**1**) is a potent compound that can be modified into a variety of the derivatives due to it have an ester functional groups which are highly reactive. Certain of the EPMC derivatives have been successfully synthesized and are known to have such biological activities[8,10-13]. Previously, Komala *et al*[10] have successfully synthesized the cinnamamide derivative of EPMC (**1**), N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) through a microwave-assisted direct amidation of EPMC (**1**) with ethanolamine[10]. This compound was reported to have *in vitro* anti-inflammatory[10] and antidepressant activity[14]. Cinnamamides are compounds that have a phenyl ring that is linked to the amide by an olefinic structure. Cinnamamide derivatives exhibit diverse of biological activity such as anticonvulsant[15], anti-depressant[14,16], antitumor[17], anti-epileptic[18], analgesic, anti-inflammatory and antimicrobial activities[19,20].

The sedative-hypnotics are a class of drugs which produce dose-dependent CNS depressant effects. Assignment of a drug into the sedative-hypnotic class is indicated by the ability of the drugs to cause sedation (with concomitant relief of anxiety) or to encourage sleep (hypnosis) [21,22]. The *n*-hexane extract of the rhizome of *K. galanga*, ethyl-p-methoxycinnamate (**1**) and ethyl cinnamate has been reported to possess sedative activity in spontaneous locomotor assay[1]. On the other report, acetone extracts of rhizome and leaf of *K. galanga* including fractions were found to have a potent CNS depressant activity in the thiopental sodium-induced sleeping time, hole cross and open field models[2]. Several cinnamamides were proved to possessed sedative-hypnotic activity, but the structure-activity relationship of the sedative-hypnotic of the cinnamamides is unclear[23]. Searching of the literature indicated that there is no any publication reported about the sedative-hypnotic activity of compound **2**. So the following study is being performed to evaluate the sedative-hypnotic activity of EPMC (**1**) and its derivatives N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) by using diazepam-induced sleeping time method.

MATERIALS AND METHODES

Plant Materials

The rhizome of *K.galanga* was collected from BALITRO (Balai Penelitian Obat dan Rempah) Bogor, West Java, Indonesia in May 2014 (No.2126/IPH.1.02/if.8/XII/2014). This species was identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

Animals

Male Sprague Dawley (SD) rats (200 to 250 g) were obtained from Badan Pengawasan Obat dan Makanan (BPOM). The animals were kept in the animal transit room, Faculty of Medicine, Syarif Hidayatullah State Islamic University at 23°C and 40-60% relative humidity in a 12-hour dark/light cycle. The experimental procedures were also performed in the same room. The animals were provided free access to water and food. The experimental procedure and the use of animals were approved by the Animal Ethics Committee of the University of Indonesia (No: 57/UN2.F1/ETIK/2017)

Instrumentation

Reactions were carried out by using a microwave oven assisted (Samsung). Column chromatography was performed on silica gel 60 (0.063-0.200 mm) (Merck). Esterification product was analyzed by using GCMS GC/MS-MSD 7890A/5975C (Agilent Technologies) under the following conditions: HP-5MS capillary column (30 m x 0.25 mm ID, 0.25 µm, film thickness) held at 70 °C for 2 mins, raised to 285 °C, at a rate of 20 C/min and held for 20 mins, injection temperature: 250 °C, 285 °C for MSD, carrier helium at a flow rate 1.2 mL/min.

Procedure

Extraction, isolation, and conversion of EPMC (1) into N-(2-hydroxyethyl)-p-methoxycinnamamide (2)

Extraction, isolation of EPMC (**1**) from the rhizome of *K. galanga* and synthesis of N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) were carried out in accordance with the previous reports[10].

Diazepam-Induced Sleep in Rats

Test samples (compound **1** and **2**) in a variety of doses (100, 200 and 400 mg/kg n=5) were administered orally to the rats, Thirty minutes later, diazepam (20 mg/kg, i.p.) was administered to each rat to induce sleep. The animals were observed for the latent period (time between diazepam administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of reflex). Diazepam at a dose of 20 mg/kg was used as a control. Percentage of effect was calculated using the following formula:

Effect % = The average duration of loss of righting reflex in the test group / The average duration of loss of righting reflex in the × 100

Statistical Analysis

The statistical analysis was conducted using ANOVA followed by non-parametric tests of Kruskal-Wallis. The results obtained were compared with the control group. $P < 0.05$ and $P < 0.01$ were considered be statically significant. The data are expressed as mean ± SD.

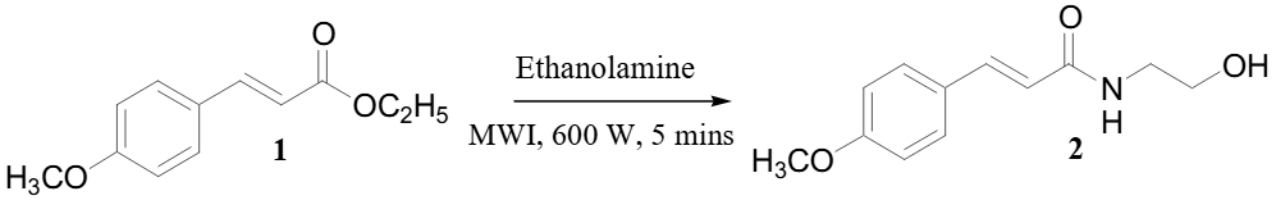

Figure 1: Scheme of the synthesis of 2 from 1

Table 1: The onset and sleep duration of Compound 1 and 2

Groups	Average (minutes)		Effect (%)
	Onset sleep	Sleep duration	
Control (Diazepam 20 mg/kg)	22.8 ± 2.4	40.8 ± 11.4	100
Compound **1** (100 mg/kg)	14.0 ±1.9	49.6 ± 7.8	121
Compound **1** (200 mg/kg)	15.8 ± 1.3	91.0 ± 8.7	223
Compound **1** (400 mg/kg)	11.8 ± 2.2	77.4 ± 17.5	189
Compound **2** (100 mg/kg)	41.2 ± 0.8	28,6 ± 13.9	-
Compound **2** (200 mg/kg)	33.2 ± 10.1	22.8 ± 9.1	-
Compound **2** (400 mg/kg)	10.8 ± 0.8	17.2 ± 11.3	-

Note: n=5, (-) = % effect lower than control

RESULTS AND DISCUSSION

Isolation of EPMC (1) and structural Modification

The EPMC (**1**) has been successfully isolated from *n*-hexane of the rhizome of K. *galanga*, which is subsequently modified to form N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**). Scheme of the synthesis is given in Figure 1. The structures of both **1** and **2** were confirmed by comparing their spectroscopic data with the previous report[8,10]. Furthermore, the sedative-hypnotic activity of both compounds was evaluated by using diazepam-induced sleep test.

Diazepam-induced sleeping test

Previously, EPMC (**1**) has been suggested to have sedative-hypnotic activity due to it showed suppression of activity in the spontaneous locomotor assay. In our study, we evaluate the sedative-hypnotic activity of EPMC (**1**) and its cinnamamide derivative, N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) by using the diazepam-induced sleeping test. Result of assay indicated that compound **1** showed significant decrease onset of sleep at a dose of 100, 200 and 400 mg/kg ($P < 0.05$), and a significant increase in duration of sleep at a dose of 200 and 400 mg/kg ($P < 0.05$), compared to the control group (Table 1).This result indicated that compound **1** beneficial in both sleep initiation and maintenance of sleep. Interestingly, at a dose of 100 mg/kg, onset sleep of rats that have been given of **1** was significantly different compared to the control, but its duration of sleep was not significantly different compared to the control. At a dose of 200 and 400 mg/kg, both onset sleep and duration of sleep showed a significant difference compared to the control. Especially at a dose of 400 mg/kg, even though, onset sleep and duration of sleep was significantly different to the control, there was a tendency a decrease of sedative-hypnotic activity if compared to a dose of 200 mg/kg. Hence, it suggested that compound **1** have a dose depending sedative-hypnotic properties in the diazepam-induce sleep assay. This result further supported the previous research which suggested that compound **1** has a sedative effect in the spontaneous locomotor assay[1].

In the contrast result, compound **2** shows significant increase onset sleep at doses of 100, 200 mg/kg ($P < 0.05$), and a significant decrease in duration of sleep at a dose of 100, 200 and 400 mg/kg ($P < 0.05$), compared to control group. Instead of showing a sedative-hypnotic effect which is indicated by the decrease of onset of sleep and increase of the duration of sleep compared to the control, compound **2** actually result in increased onset of sleep and decrease the duration of sleep.

Hence, it suggests that there is a possibility that compound **2** to have a stimulant effect rather than sedative effect. The interesting result showed at a dose of 400 mg/kg of compound **2**, which showed a significant decrease of onset of sleep ($P<0.05$) and increase the duration of sleep ($P<0.05$). This makes a possibility to suggest that compound **2** at a dose of 400 mg/kg is effective as a sedative but not active as hypnotic. Previously, compound **2** has been reported significantly reduced the immobility time in the tail suspension test (TST) which suggested to have an antidepressant-like action[14]

CONCLUSION

EPMC (**1**) is a major component detected in the rhizome of the *K. galanga* and suggest to be responsible for the sedative-hypnotic activity of this plant. In this research, we supported the previous reported which indicated that EPMC has a sedative activity. We found that EPMC at a dose of 200 and 400 mg/kg showed significant sedative-hypnotic activity. In the contrast result, the N-(2-hydroxyethyl)-p-methoxycinnamamide (**2**) did not show sedative-hypnotic activity.

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