

The Effect of Co-Administration of *Telang* Leaves Ethanolic Extract Towards Fluoxetine's Sedative Effect on Male Balb/C Mice Based on Sleeping Duration Parameter

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Abstract

One of *Telang* plant's advantages is its activity as sedative agent. Previous studies indicate that *Telang* plant has anticonvulsant activity on mice at certain dose. This study was conducted to find out the sedative effect of *Telang* plant, particularly its leaf, when being co-administered with fluoxetine. Phytochemical analysis was carried out qualitatively on *Telang* ethanolic extract leaves (TEE) to find out the content of chemical compound first. This study was an experimental research with post-test only control group design, employing male balb/c mice. The effect of *Telang* ethanolic extract co-administration with fluoxetine was observed. The parameter being used in the study was the duration of sleep. Analysis was done by comparing mice sleeping duration prior to administration of fluoxetine alone and in combination with *Telang* ethanolic extract. The data was then analyzed using SPSS 17.0 for Windows. The results showed that TEE contained tannin, saponin, and flavonoid compounds, and co-administration of TEE and fluoxetine at various doses could provide sedative effect on mice. The co-administration of 400 mg/kgBW extract and 15 mg/kgBW fluoxetine could provide the mean sleeping duration 43 minutes longer than positive control.

Keywords: *telang* leaves ethanol extract, fluoxetine, sleeping duration.

INTRODUCTION

One of the application of herbal medicine is to treat sleeping disorder. It was estimated that in every year, there were as many as 20-40% adults having chronic sleep and wakefulness sleeping disorders (National Sleep Foundation, 2011). The prevalence of sleeping disorder every year has been increasing, caused by aging and other causes. Based on Indonesian Department of Health, the number of older society having sleeping disorder each year reaches 750 people. Insomnia is the most prevalent sleep disorder among adults. The estimated prevalence of difficulty in initiating and maintaining sleep is about 30% (LeBlanc, *et al.*, 2007). Regarding to those problems, *Telang* plants (*Clitoria ternatea* L.) is known to have some benefits.

In several regions in Indonesia, this plant is traditionally used as laxative, diuretic, anthelmintic, intelligence-promoting, and anti-

inflammatory properties and they are useful in severe bronchitis, asthma and dementia, hemicrania, burning sensation, leprosy, inflammation, leucoderma, pulmonary tuberculosis, ascites, and hectic fever (Mukherjee, *et al.*, 2008). The leaves and roots of *Telang* plants are used as the medicine for several diseases, such as peripheral pain, infection, and urogenital disturbance (Patil and Patil, 2011). *Telang's* roots extract is used in the medication of amnesia (Ravishankar and Parvathi, 2012). *Telang* plant is reported to possess some pharmacological activities, such as antimicrobial, antipiretic, antiinflammation, analgesic, antidiabetic, insecticide, and relaxant (Mukherjee, *et al.*, 2008).

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Several previous studies reported that TEE showed the modulation of obsessive-compulsive behaviour in mice which have the comparable effect to fluoxetine in the dose range of 5, 10, and 15 mg/kgBW (Shende, *et al.*, 2012).

Ethanol extract of *Telang* in the dose of 100, 200, and 499 mg/kgBW did not induce sedative activity, but higher dose showed the potential of sedative activity (Kulkarni, 1998). Therefore, in this paper, we studied the sedative effect of TEE if it is co-administrated with fluoxetine in order to reduce the therapeutic dose of fluoxetine. Besides, the initial qualitative study of the compounds contained in this extract was also conducted.

MATERIALS AND METHODS

Ethanol extract of *Telang* Leaves (TEE)

As many as one kilogram of *Clitoria ternatea* leaves is obtained from Rejosari village, Madiun, Jawa Timur, Indonesia. The leaves were cleaned, then dried by using oven in the temperature of 50°C and powdered. The extraction of powder was done by ethanol 70%, and evaporated by rotary evaporator.

Qualitative Phytochemistry Assay for Alkaloid Content

As many as 2 mL of TEE was added by 1 mL of HCL 2 N and 9 mL of hot water, and heated in the temperature of 100°C for 2 minutes. Then the mixture was cooled down and filtrated. Approximately 3 drops of filtrate were added by Wragener reagent on the glass and the formation of brown sediment as a sign of the existence of alkaloid was observed.

Qualitative Assay for Tannin Content

Approximately 2 mL of TEE was added by 10 mL of hot water, heated in the water incubator in the temperature of 100°C for an hour, and filtrated as it is cooled down. Filtrate was dropped by 1% Iron (III) chloride. The blue-green or black colour showed the existence of tannin.

Qualitative Assay for Saponin Content

As many as 2 mL of TEE were added by 10 mL hot water, then it was cooled and shaken for

10 minutes or until stable foam was formed as high as 1-10 cm. Saponin content is shown by the existence of the foam after the addition of 2 N HCl.

Qualitative Assay for Flavonoid Content

As many as 2 mL of TEE were added by the powder of Mg, 1 mL HCl:ethanol 70% (10:10), and some amyl-ethanol until two layers were formed. Positive result is showed by the color of layer become yellow, bright red, or red.

Qualitative Assay for Triterpenoid Content

As many as 2 mL of TEE were added by ethanol 70% and heated, and filtered. Filtrate then was evaporated and added by ether. The ether layer was taken and dropped by Liebermann-Burchard reagent (3 drops of Acetic acid anhydrate + 1 drop of H₂SO₄). Positive result is shown by the formation of green to blue color.

In Vivo Assay for Sedative Effect of TEE

As many as 18 balb/c male mice were used in the treatment. The tested animals were divided into 6 groups, each of which consisted of 3 mice. Group I was negative control given CMC p.o., group II was positive control given 20 mg/kgBW fluoxetin i.p., group III was given 100 mg/kgBW TEE p.o. and 5 mg/kgBW fluoxetin i.p., group IV was given 200 mg/kgBW TEE p.o. and 10 mg/kgBW fluoxetin i.p., group V was given 500 mg/kgBW TEE p.o. and 15 mg/kgBW fluoxetin i.p., and group VI was given 400 mg/kgBW TEE p.o. The sedative effect were analyzed by the observation of the sleeping duration of the mice.

Data Analysis

Data were analyzed by SPSS 17.0 for windows one Way ANOVA (Analysis of Variance) continued by *Post Hoc Test* ($p < 0.05$) to define the significance of differences between groups.

RESULTS

The results of this study are shown below (Fig. 1, Fig. 2, and Table 1).

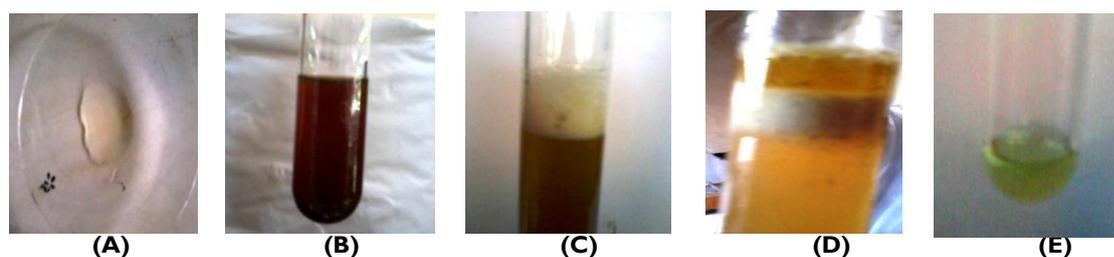
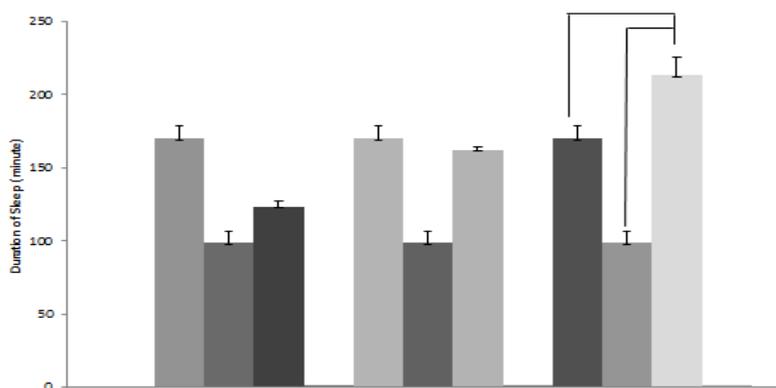


Figure 1. The result of Qualitative Phytochemistry Assay. It showed that the TEE contain no alkaloid by showing no brown precipitate (A), meanwhile the Tannin assay showed positive result by forming black color solution (B), stable foam until ± 10 minutes (1.5 cm) showed the existence of Saponin (C), Flavonoid content showed by two layers having the color yellow to reddish yellow (D), and green color showed the positive result for Triterpenoid content (E).

Table 1. The effects of TEE on sleeping duration of mice

Replication	Sleeping duration of each groups (minutes)					
	Negative control	Positive control	Fluoxetine 5 mg/kgBW + TEE 100mg/kgBW	Fluoxetine 10 mg/kgBW + TEE 200mg/kgBW	Fluoxetine 15 mg/kgBW + TEE 400mg/kgBW	TEE 400 mg/kgBW
1	0	162	120	160	200	90
2	0	167	122	163	215	100
3	0	180	128	164	224	105
Mean\pmSD	0	169.67 \pm 9.292	123.33 \pm 4.163	162.33 \pm 2.082	213.00 \pm 0.02	98.33 \pm 7.638



Fluoxetine 20 mg/kg BW	+	-	-	+	-	-	+	-	-
TEE 400 mg/kg BW	-	+	-	-	+	-	-	+	-
Fluoxetine 5 mg/kg BW	-	-	+	-	-	-	-	-	-
TEE 100 mg/kg BW	-	-	+	-	-	-	-	-	-
Fluoxetine 10 mg/kg BW	-	-	-	-	-	+	-	-	-
TEE 200 mg/kg BW	-	-	-	-	-	+	-	-	-
Fluoxetine 15 mg/kg BW	-	-	-	-	-	-	-	-	+
TEE 400 mg/kg BW	-	-	-	-	-	-	-	-	+

Figure 2. Result of sedative effect observation by using sleep duration as the parameter. The Table showed a significant difference between positive control and co-administration of Fluoxetine and TEE.

DISCUSSION

Phytochemical screening is a qualitative method used to determine the secondary metabolites of crude extract. This study showed that *Telang* leaves contains tannin, saponin, flavonoid, and triterpenoid.

Sedative effect was reviewed from mice's sleeping time duration after received treatment. The duration was started once sedative effect was shown and finished when the mice woke up and could do some activities as before-treatment phase. This study showed that higher co-administration fluoxetine and TEE dose increased sleeping time of mice.

For each treatment and test, separate groups of mice were used. Mice were injected with saline (10 mL/kgBW, p.o.), fluoxetine, and/or TEE as describe above and individual mouse was observed for sleeping time duration from locomotor activity. One-way ANOVA indicated the significant influence of TEE (100, 200, and 400 mg/kgBW, p.o.) dose-dependently on sleeping time duration compared to control. Newman-keuls test indicated that TEE (100, 200, and 400 mg/kgBW, p.o.) decreased marble-burying behavior in mice in a dose-dependent manner. Figure 1 and figure 2 showed that TEE did not affect motor activity compared to the saline treated group ($p>0.05$).

Post Hoc Test showed no significant difference between positive control and co-administration of fluoxetine 5 mg/kgBW and also TEE 100 mg/kgBW (group I) ($p>0.05$). Co-administration of 10 mg/kgBW fluoxetine and 200 mg/kgBW TEE (group II) also showed no significant differences ($p>0.05$). Co-administration of 15 mg/kgBW fluoxetine and 400 mg/kgBW TEE (group III) and 400 mg/kgBW TEE (group IV) showed significant differences ($p<0.05$) compared to positive control which means group IV gave different sedative effect to positive control. The combination of TEE 400 mg/kgBW and fluoxetine 15 mg/kgBW gave sedative effect more than positive control.

CONCLUSION

Qualitative phytochemical screening showed that TEE contains tannin, saponin, and flavonoid. TEE administrated with the dose of 400 mg/kgBW followed by 15 mg/kgBW fluoxetine performed sedative effect with average of sleeping time duration was 43 minutes longer than single administration of 400 mg/kgBW fluoxetine.

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