PHARMACODYNAMIC IDENTIFICATION OF ANTISEIZURE EFFECT OF LIGUSTROSID GLYCOSIDE (A CNS ACTIVE SUBSTANCE) ISOLATED FROM Fraxinus griffithii Clarke ON MICE

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ABSTRACT

A study was conducted to prove whether Ligustrosid (A CNS Active substance) isolated from bark of Fraxinus griffithii Clarke has antiseizure effects. Antiseizure test showed that Ligustrosid increased seizure threshold on epileptic animals injected by CD 90 Metrazol i.p. (ED 50 Anti Met : 278.54 mg/kg BW), and prevented seizure spread (ED 50 Anti M.E.S : 431.61 mg/kg BW). Furthermore, these experiment reported that Ligustrosid decreased Metrazol lethality test on animals. The capability of Ligustrosid to increase the seizure threshold against Anti Metrazol test meant that Ligustrosid clinically would be active against absence seizures and myoclonic seizures. It is strongly supposed that Ligustrosid may has antiseizure effects by increasing GABA-facilitated mechanisms of Ligustrosid stabilizing membrane through interaction with Sodium channel receptors. Further research will be needed to explore antiseizure to increase the seizure threshold against Anti Metrazol test meant that Ligustrosid clinically would be active against absence seizures and myoclonic seizures. It is strongly supposed that Ligustrosid may has antiseizure effects by increasing GABA-facilitated mechanisms of Ligustrosid stabilizing membrane through interaction with Sodium channel receptors. Further research will be needed to explore antiseizure effects. Antiseizure test showed that Ligustrosid increased seizure threshold on epileptic animals injected by CD 90 Metrazol i.p. (ED 50 Anti Met : 278.54 mg/kg BW), and prevented seizure spread (ED 50 Anti M.E.S : 431.61 mg/kg BW). Furthermore, these experiment reported that Ligustrosid decreased Metrazol lethality test on animals. The capability of Ligustrosid to increase the seizure threshold against Anti Metrazol test meant that Ligustrosid clinically would be active against absence seizures and myoclonic seizures. It is strongly supposed that Ligustrosid may has antiseizure effects by increasing GABA-facilitated mechanisms of Ligustrosid stabilizing membrane through interaction with Sodium channel receptors. Further research will be needed to explore antiseizure effects. Antiseizure test showed that Ligustrosid increased seizure threshold on epileptic animals injected by CD 90 Metrazol i.p. (ED 50 Anti Met : 278.54 mg/kg BW), and prevented seizure spread (ED 50 Anti M.E.S : 431.61 mg/kg BW). Furthermore, these experiment reported that Ligustrosid decreased Metrazol lethality test on animals. The capability of Ligustrosid to increase the seizure threshold against Anti Metrazol test meant that Ligustrosid clinically would be active against absence seizures and myoclonic seizures. It is strongly supposed that Ligustrosid may has antiseizure effects by increasing GABA-facilitated mechanisms of Ligustrosid stabilizing membrane through interaction with Sodium channel receptors. Further research will be needed to explore antiseizure effects.

Keywords: Ligustrosid, Anti MES, Anti Met, antiseizure effect

INTRODUCTION

Fraxinus griffithii Clarke (trees: 10 - 20 m) usually grows in forest margins, near villages, by rivers; 100-2000 m in several regions in Bangladesh, India, Ryukyu Islands, Myanmar, Philippines, Vietnam and Indonesia. In Java island, Fraxinus griffithii Clarke is known as Pohon Tiken (Kabupaten Lumajang), Bedali Gombong (Kabupaten Probolinggo), and Pohon Orang Aring (Perkebunan Pancur Angkrek, Prabekan) (Heyne, 1987). Extract of the bark and the leaves of Fraxinus griffithii Clarke (so called: ektrak Tiken) have been used as an adulterant of illegal opium in certain areas in Indonesia (Boerlage dan Kooders, 1987; Heyne, 1987; Sutarjadi and Norcholis, 1973; Wehmer, 1931). Phytochemical studies found that Tiken extract contains saponin, tannine, and glycosides, and no alkaloids (Sutarjadi and Norcholis, 1973). Survey in 1975 reported that the workers who prepared this extract felt sedation and sometimes went to sleep (Ahaditomo, 1975). Explorative study in mice showed that Tiken extract caused little sedation, and reduced motor activity (Ma'rifin, 1975). Furthermore, Basori et al (1998) found that Tiken extract contained 1500 mg/kg BW up to 2500 mg/kg BW given orally to animals caused little sedation, reduced locomotor activity, did not cause coordination disturbances and muscle paralysis, and did not have hypnogenic effects. All those findings strongly suggested that Fraxinus griffithii Clarke may contain a CNS (Central Nervous System) active substance.

In 1980, Sutarjadi modified the method of Inouye (1975) to isolate Ligustroside glycoside from the bark of Fraxinus griffithii Clarke. For further research and development of Ligustroside to be lead compound of CNS depressant drug, a large amount of Ligustroside and high quality is needed. Basori (1997) modified the method of Sutarjadi to isolate Ligustroside by doing pre-extraction process with dichloromethane. This modification proposed to remove all non-glycoside compounds from the solution and expected to give a more pure Ligustrosid.

The previous pharmacodynamic screening showed that Ligustroside at doses of 100 mg/kg, 200 mg/kg , and 400 mg/kg given intraperitoneally to mice caused little sedation, reduced locomotor activity, did not cause motor coordination disturbances and muscle paralysis, and did not have hypnogenic effect. These results suggested that Ligustroside may have CNS activity as CNS depressant (Basori, 1999). But the precise pharmacodynamic profile of its CNS depressant has not been studied in detail yet. Further research is needed to explore pharmacodynamic profile of its CNS depressant effect.

A very detailed pharmacodynamic study was conducted by Basori (2000), to explore the profile of CNS depressant effects of Ligustroside. Those studies concluded that Ligustrosid has pharmacodynamic activity as CNS depressant, did not have neuromuscular blocking properties. The primary site of action of its
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CNS depressant is in the brain, and the mechanism of action may involve the enhancement of GABA-facilitated inhibition in the brain neurons.

The present research aims to identify whether Ligustrosid isolated from bark of *Fraxinus griffithii Clarke* has antiseizure effects.

**MATERIALS AND METHODS**

**Materials**

Ligustrosid

Material used for the present work was dried bark of *Fraxinus griffithii Clarke* (Pohon Orang Aring). Wet bark was collected in May 1997 from plants used as shade trees at a Pancur Angkrek coffee plantation of PTP XXVI located near Prajekan, East Java. The bark was cut into pieces and dried at room temperature, avoiding direct sunlight, for 1 month. This was done in order to keep active substances from degradation by direct sunlight. Later, the dried bark was milled with a milling machine into smaller particles, sieved into pulves, and stored in tightly sealed plastic bottles. Ligustrosid was isolated, identified and purified according to the previous method (Basori, 1999).

Animals

Male BALB/c mice (25-30 g, age: 2 months) used in all experiments were purchased from Pusvetma, Surabaya. Animals were acclimatized for at least one week before starting the experiments. They were kept in a soundless room with normal room temperature. During the experiments the animals were not allowed to drink or eat. Finally, the animals were given a code for experiments.

Drugs and dosages

The following drugs and dosages were used: Ligustrosid 100, 200, 300, 400 and 500 mg/kg. This drug was dissolved in solutio Petit (10% absolut alcohol;20% Propylen glycol : 80 % water pro injection) and administrated to animals by i.p. injection. Control animals (positive and negative control) were dosed with Solutio Petit and water pro injection. The previous study found that solutio Petit did not have antiseizure effects (Basori, 2000).

**Antiseizure Identification**

Determination of Time Peak Effects (TPE) of Ligustrosid

The animals were randomly divided into 2 groups (each group consists of 20 mice). Each group of animals were given Ligustrosid 400 mg/BW intraperitoneally. Thirty minutes after Ligustrosid administration, group I was given CD 90 Metrazol intraperitoneally (Convulsive Dose 90 Metrazol : 66.75 kg/BW i.p.). Forty minutes after Ligustrosid administration, group II was given CD 90 Metrazol intraperitoneally. The ED 50 value of anti-Metrazol effects was calculated according to the Probit method by using SPSS 9. Time Peak Effect (TPE) means the time at which Ligustrosid reaches maximal concentration in the body.

Effect of Ligustrosid against Maximal Electroshock Seizure (MES)

The animals were randomly divided into 4 groups (each group consists of 20 mice). Each group of animals were given Ligustrosid 200 mg/kg, 400 mg/kg, and 500 mg/kg i.p. Animals were placed in individual transparent plastic cages. Forty minutes after given Ligustrosid, a 60 Hz alternating current of 50 mA was applied to the animals through corneal electrodes for 0.1 second with maximal electroshock seizure apparatus. Electroconductivity was enhanced with two drops of 0.9% NaCl on each eye. Measurements were done against seizure patterns (clonic flexion, tonic hind leg extension, and clonic). The main measurable component of this experimental model is tonic hind-leg extension (THE) (Krall, 1978; Swinyard, 1972; Swinyard et al, 1985; Leppik, 1993, 1994; White,HS et al, 1995). Abolition of tonic hindleg extension after drug treatment was regarded as the end point of protection. In this experiment, the dose - responses curves were estimated by testing three doses and twenty animals per dose. The calculation of ED50 anti MES (a dose that protects 50% of the animals against electroshock-induced tonic hindleg extension), and the statistical analyses were performed according to Probit methods by using SPSS 9.

Effect of Ligustrosid against Metrazole Induced Seizure (Met test)

The animals were randomly divided into 4 groups (each group consists of 20 mice). Each group of animals were given Ligustrosid 100 mg/kg BW, 200 mg/kg BW, 300 mg/kg BW, and 400 mg/kg BW i.p. Animals were placed in individual transparent plastic cages. Forty minutes after given Ligustrosid, all animals were injected with Metrazole 66.75 mg/kg BW i.p (Convulsive Dose 90). The capability of a substance to protect animals against clonic seizure regarded as anti-
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Metrazole activity (Anti Met effect) (Krall, 1978; Swinyard, 1972; Swinyard et al, 1985; Leppik, 1993, 1994; White, HS et al, 1995). In this experiment, the dose - responses curves were estimated by testing four doses and twenty animals per dose. The calculation of ED50 anti Metrazol (a dose that protects 50% of the animals against Metrazol induced clonic seizure), and the statistical analyses were performed according to Probit method by using SPSS 9.

RESULTS AND DISCUSSIONS

The specific objective of these pharmacodynamic study was to identify antiseizure effect of Ligustrosid. The basic experimental methods used in this research were Maximal Electroshock Seizure test (MES test) and Metrazole induced seizure test (Metrazol test). In this experiment, exploration study found that Time Peak Effects (TPE) of Ligustrosid was 45 minutes (Table 1). Furthermore, injection of chemical epileptogen (CD 90 Metrazol) and electrical stimulation through cornea (50 mA, 60 Hz, 1 second) were applied to animals at 45 minutes after Ligustrosid administration.

In order to prove whether Ligustrosid has capability to increase seizure threshold of epileptogenic foci, an experimental of anti Metrazol test was done. The results showed that Ligustrosid has capability to prevent clonic seizures on animal and to decrease mortality rate on animals after given CD 90 (Convulsive Dose 90) Metrazol intraperitoneally (table 2). The ED 50 value of Anti Metrazol effects of Ligustrosid was 278.4 mg/kg BW (Table 4). Clinically, drugs that has anti Metrazol effect would be active against absence seizures and myoclonic seizures (Rogawski, 1991; White et al, 1995; Porter and Meldrum, 2001).

In order to prove whether Ligustrosid has capability to inhibit seizures spreads from epileptogenic foci to other normal neuronal population, an experimental of anti MES (Maximal Electroshock Seizures) was done. The results showed that Ligustrosid has an effect to inhibit Tonic Hindleg Extension (THE) on animals (table 3). The ED 50 value of anti MES of Ligustrosid was 431.61 mg /kg BW (table 4). Clinically, drugs that has anti MES effects would be active against generalized tonic clonic seizures and partial seizures (Rogawski, 1991; White et al, 1995; Porter and Meldrum, 2001).

Finally, it was proved pharmacologically that Ligustrosid had antiseizures effects on animals, and can be developed further to be antiseizure drug candidate. It was strongly suggested that Ligustrosid may has antiseizure effects by interaction with Benzodiazepine receptor from GABA-Benodiazepine Receptor Complexes and by blocking Sodium channel activation on neuron in CNS (Rogwaski, 1991; Gale, 1992; MacDonald, and Kelly, 1993, 1994; Ramsay and Slater, 1993; White, 1995; Porter and Meldrum, 2001). Further research will be needed to explore deeply antiseizure mechanism of Ligustrosid.

<table>
<thead>
<tr>
<th>Table 1. Antiseizure effects on mice after given Ligustrosid 400 mg/kg BW and CD 90 Metrazol intraperitoneally</th>
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<tbody>
<tr>
<td>Times after Ligustrosid administration (minutes)</td>
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<td>30</td>
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<td>45</td>
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<tr>
<th>Table 2. Effects of Ligustrosid against seizure threshold on mice after given CD 90 Metrazol intraperitoneally</th>
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<tr>
<td>Dosage of Ligustrosid (mg/kg BW, i.p)</td>
</tr>
<tr>
<td>100</td>
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<tr>
<td>200</td>
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<tr>
<td>300</td>
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<td>400</td>
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Tabel 3. Effects of Ligustrosid against seizure spread on mice after stimulated by supramaximal currents (50 mA, 60 mHz, 1 second)

<table>
<thead>
<tr>
<th>Dosage of Ligustrosid (mg/kg BW, i.p)</th>
<th>No. of mice without T.H.E / No. of total mice</th>
<th>Antiseizure spread (%)</th>
<th>No. of dead mice / No. of total mice</th>
<th>Mortality rate (%)</th>
</tr>
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<tbody>
<tr>
<td>200</td>
<td>0 / 20</td>
<td>0</td>
<td>4 / 20</td>
<td>20</td>
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<tr>
<td>400</td>
<td>10 / 20</td>
<td>50</td>
<td>0 / 20</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>12 / 20</td>
<td>60</td>
<td>0 / 20</td>
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Table 4. ED 50 value of anti Metrazol effects and anti M.E.S effects of Ligustrosid on mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Anti Metrazol (mg / kg BW, ip)</th>
<th>ED 50</th>
<th>Anti M.E.S (mg / kg BW, ip)</th>
<th>ED 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligustrosid</td>
<td>278.54</td>
<td>431.61</td>
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CONCLUSION
Pharmacodynamic study with chemical epileptogen and electrical stimulation indicated that Ligustrosid has an effects to increase seizure treshold and to prevent seizure spreads on animals. Pharmacologically, Ligustrosid was proved to has antiseizure effects on animals. In another world, Ligustrosid was very potential to be developed as antiseizure drug candidate. Further research will be needed to investigate deeply the antiseizure mechanism of Ligustrosid.

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