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

Achmad Basori

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 treshold 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 treshold 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 inhibition in CNS through interaction with Benzodiazepine receptor of GABA-BZD-BARB/PICRO-CL receptor complexes. The capability of Ligustrosid to prevent seizure spread against Anti M.E.S test meant that Ligustrosid clinically would be active against generalized tonic clonic seizures and partial seizures. It is strongly supposed that ligustrosid may has antiseizure effects by stabilizing membrane through interaction with Sodium channel receptors. Further research will be needed to explore antiseizure mechanisms of Ligustrosid*

***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, Prajekan) (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 Noorcholis, 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 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.

Department of Pharmacology
Airlangga University School of Medicine

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 Ligustrosid by doing pre extraction process with dichlormethane. 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

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 aimes to identify wether 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 griffithi 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 on, the dried bark was milled with a milling machine into smaller particles, sieved into pulves, and stored in tighly sealed plastic bottles. Ligustrosid was isolated, identified and purified according to the previous method (Basori, 1999)

Animals

Male BULB/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 groups 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). Fourty 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 groups of animals were given Ligustrosid 200 mg/kg, 400 mg/kg, and 500 mg/kg i.p. Animals were placed in individual transparant plastic cages. Fourty 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 electroschock 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 groups 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 transparant plastic cages. Fourty 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 -

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 Electroschock 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 treshold 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 Electroschock 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-Benzodiazepine 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 reseach will be needed to explore deeply antiseizure mechanism of Ligustrosid.

Table 1. Antiseizure effects on mice after given Ligustrosid 400 mg/kg BW and CD 90 Metrazol intraperitoneally

Times after Ligustrosid administration (minutes)	(no. of seizure mice / no. of total mice)	Antiseizure effects (%)
30	8 / 20	60
45	2 / 20	90

Table 2. Effects of Ligustrosid against seizure treshold on mice after given CD 90 Metrazol intraperitoneally

Dosage of Ligustrosid (mg/kg BW, i.p)	No. of seizure mice / no. of total mice	Antiseizure treshold (%)	No. of dead mice / no. of total mice	Mortality rate (%)
100	20 / 20	0	14 / 20	70
200	16 / 20	20	8 / 20	40
300	10 / 20	50	2 / 20	10
400	2 / 20	90	0 / 20	0

Tabel 3. Effects of Ligustrosid against seizure spread on mice after stimulated by supramaximal currents (50 mA, 60 mHz, 1 second)

Dosage of Ligustrosid (mg/kg BW, i.p)	No. of mice without T.H.E / No. of total mice	Antiseizure spread (%)	No. of dead mice / No. of total mice	Mortality rate (%)
200	0 / 20	0	4 / 20	20
400	10 / 20	50	0 / 20	0
500	12 / 20	60	0 / 20	0

Table 4. ED 50 value of anti Metrazol effects and anti M.E.S effects of Ligustrosid on mice

Drug	ED 50 Anti Metrazol (mg / kg BW, ip)	ED 50 Anti M.E.S (mg / kg BW, ip)
Ligustrosid	278.54	431.61

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.

REFERENCES

Ahaditomo, Suwandi A, and Ma'rifin Husin D, 1975. Penelitian pendahuluan beberapa khasiat tiken (*Fraxinus Griffithii Clarke*). Proseding Simposium penelitian tanaman obat I , Bogor, 267 - 398

Ahaditomo, 1987. Pengaruh eksktrak tiken (*Fraxinus griffithii Clarke*) pada saraf simpatis melalui efeknya pada membran nictitans kucing, Thesis S2, Program Pasca sarjana, Unair, 1987

Basori A and Prayogo B, 1997 Modifikasi metode isolasi Ligustrosid dari *Fraxinus griffithii Clarke* dengan menggunakan Dichlormethane (Penelitian yang tidak dipublikasi).

Basori A:, 1999. Isolation and Pharmacodynamic Screening of Ligustroside (A CNS active Substance) isolated from *Fraxinus griffithii Clarke*, Folia Medica Indonesiana (4) October - December, p. 4 - 8.

Basori A, 2000. Pharmacodynamic studies of Central Nervous System Depressant Effects of Ligustrosid (A CNS Active Substance) isolated from *Fraxinus griffithii Clarke*, Folia Medica Indonesiana (1) January - March, p 39 - 46

Boorlage JG and Koorders SH, 1987. Een Nieuwe Javaasche Boomsort, Natuurke Tijdshr, Ned\.In, 1987, p 56, 158 - 189

Gale K, 1992. GABA and Epilepsy:basic Concepts from Preclinical Reseach, Epilepsia 33 (Suppl.5) S3-S12.

Heyne K, 1987 : Tumbuhan Berguna Indonesia, Jilid III, Cetakan I, Badan Litbang Kehutanan Jakarta, Jakarta, halaman 1611

Krall RJ, 1978. Antiepileptic Drug Development, In: History and Program for Progress, Epilepsia 19: 393 - 408

Leppik IE, 1993. Antiepileptic Drug in Development, prospect for the near future. Epilepsia 34: S1 - S8.

Leppik IE, 1994. Antiepileptic Drugs in development:Prospects for the near Future, Epilepsia 35 (Suppl. 4): SS29-S40.

MacDonald RL and Kelly KM, 1993. Antiepileptic Drug mechanisms of Action, Epilepsia 34 (Suppl. 5):S1-S8.

MacDonald RL and Kelly KM, 1994. Mechanisms of Action of Currently Prescribed and Newly Developed Antiepileptic Drugs, Epilepsia 35 (Suppl. 4): S41-S50.

Porter RJ, 1993. Antiepileptic Drugs Future Development, In: New Antiepileptic Drug Development, Preclinical and Clinical Aspects, edited by French, JA, Dichter MA, dan Leppik IE, Elsevier, Netherlands, 1993, p 69 - 80

Porter RJ, 1993. Antiepileptic drugs: future development, In: New Antiepileptic Drug Development, Preclinical and Clinical Asspects,

edited by: French JA, Dichter MA, and Leppik IEE; Elsevier, Netherlands, pp. 69 - 80.

Porter RJ and Rogawski MA, 1992. New Antiepileptic Drugs:From Serendipity to Rational Discovery, Epilepsia 33 (Suppl.1): S1-S6.

Porter RJ and Meldrum BS, 2001. Antiseizure Drugs, In: Basic and Clinical Pharmacology, edited by BG Katzung, 8th edition, Lange Medical Books, NY, p 395 - 417.

Ramsay RE and Slater JD, 1993. Antiepileptic drugs in clinical development. In: New Antiepileptic Drug Development, Preclinical and Clinical Aspects, edited by French JA, Dichter MA and Leppik IE: Elsevier, Netherlands, p45-p68.

Ramsay RE and Slater JD, 1993. Antiepileptic Drugs in Clinical Development, In : New Antiepileptic Drug Development, Drug Development, edited by French, JA, Dichter MA and Leppik IE, Elsevier, Netherlands, 1993, p 45 - 68, 1993

Rogawski MA and Porter RJ, 1991. Antiepileptic Drugs: Pharmacological Mechanisms and Clinical Efficacy with Consideration of promising Developmental Stage Compounds, Pharmacol Rev, 42:224-277.

Sutarjadi and Noor Cholies Zaini, 1973. Fraxinus eedenii Boerl. Et Kds. (Studi Pendahuluan Farmakognosi dan Fitokimia). Laporan Penelitian Universitas Airlangga, 676

Sutarjadi, 1980 *Fraxinus griffithii Clarke*, Penelitian taksonomi dan Fitokimia, Disertasi, Universitas Airlangga.

Swinyard EA et al, 1989. Experimental Selection, Quantification, and Evaluation of Anticonvulsants. In: Antiepileptic Drugs, edited by Levy RH et al 3th edition, Raven Press, NY, p 85-102

Wehmer C, 1931. Die Pflanzenstoffe, Zweiterband, Jena Verlag van Gustav Fiesher, p 951.

White HS et al, 1995. Experimental Selection, Quantification, and Evaluation of Antiepileptic Drugs, In: Antiepileptic Drugs, 4th edition, edited by Rene H Levy et al, Raven Press, NY, p99 - 110.