

Evaluation of the Effect of Jobelyn® on Chemoconvulsants-Induced Seizure in Mice

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ABSTRACT

Introduction: Epilepsy is a common central nervous system (CNS) disorder characterized by seizures resulting from episodic neuronal discharges. The incidence of toxicity and refractoriness has compromised the clinical efficacy of the drugs currently used for the treatment of convulsions. Thus, there is a need to search for new medicines from plant origin that are readily available and safer for the control of seizures. Jobelyn® (JB) is a unique African polyherbal preparation used by the natives to treat seizures in children. This investigation was carried out to evaluate whether JB has anti-seizure property in mice.

Methods: The animals received JB (5, 10 and 20 mg/kg, p.o) 30 min before induction of convulsions with intraperitoneal (i.p.) injection of picrotoxin (6 mg/kg), strychnine (2 mg/kg) and pentylentetrazole (85 mg/kg) respectively. Diazepam (2 mg/kg, p.o.) was used as the reference drug. Anti-seizure activities were assessed based on the ability of test drugs to prevent convulsions, death or to delay the onset of seizures in mice.

Results: JB (5, 10 and 20 mg/kg, p.o) could only delay the onset of seizures induced by pentylentetrazole (85 mg/kg, i.p.) in mice. However, it did not offer any protection against seizure episodes, as it failed to prevent the animals, from exhibiting tonic-clonic convulsions caused by pentylentetrazole (85 mg/kg, i.p.), strychnine (2 mg/kg) or picrotoxin (6 mg/kg, i.p.). On the other hand, diazepam (2 mg/kg, p.o.), offered 100% protection against convulsive seizures, induced by pentylentetrazole (85 mg/kg, i.p.). However, it failed to prevent seizures produced by strychnine (2 mg/kg, i.p.) or picrotoxin (6 mg/kg, i.p.).

Discussion: Our results suggest that JB could not prevent the examined chemoconvulsants-induced convulsions. However, its ability to delay the latency to seizures induced by pentylentetrazole suggests that JB might be effective in the control of the seizure spread in epileptic brains.

1. Introduction

Epilepsy is a common central nervous system (CNS) disorder characterized by seizures which take diverse forms and result from episodic neuronal discharges. Meanwhile, the form of the seizure depends on the part of the brain affected (Rang et al., 2000; Leonard, 2000). Epilepsy affects 0.5-1% of the population globally (Rang et al., 2000). Often, there is no recognizable

cause, although it may develop after brain insults, such as trauma, infection, tumor growth, or other kinds of neurological disorders (Leonard, 2000).

Current anticonvulsant drugs are effective in controlling seizures in about 70% of cases; however, their use is often limited by severe side effects (Rang et al., 2000). As alternatives to these existing drugs, a number of compounds from plants that are readily available and well tolerated are being developed as potential medica-

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tions for the management of seizures (Lobo et al., 2008; WHO, 1996). Jobelyn® (JB), manufactured by Health Forever Products Ltd, Lagos, Nigeria, is a commercial herbal preparation which has recently made its way into the medicine market in Nigeria. It is available as a powdered preparation specially formulated into capsules and suspensions for the treatment of anemia (Erah et al., 2003; Okochi et al., 2003). JB is made from three notable medicinal plants; *Parquetina nigrescens*, *Harungana madagascariensis* and *Sorghum bicolor*. *Parquetina nigrescens* (family: *Periplocaceae*) is a shrub found in the equatorial region of West Africa (Mabberley, 1987). *Harungana madagascariensis* (family: *Clusiaceae*) is locally cultivated in Madagascar, Mauritius and Tropical Africa for its valuable medicinal properties (Hutchinson & Dalziel, 1954). *Sorghum bicolor* or *Sorghum* (family: *Gramineae*) is an important staple food crop in Africa, South Asia and Central America, and is also grown in some developed nations for its medicinal values (Erah et al., 2003; Okochi et al., 2003). Most of the compounds in JB including apigenin, luteolin and naringenin, have been reported to exhibit a wide range of CNS activities (Okochi et al., 2003; Olsen et al., 2008; Yi et al., 2010). Apigenin, luteolin and naringenin in particular have been shown to exhibit neuroprotection, anti-oxidation and to reduce neuroinflammation, suggesting their therapeutic efficacy in CNS disorders (Yi et al., 2010; Awika & Rooney, 2004).

Jobelyn® is claimed to be helpful in stress related ailments and has gained international recognition as energizer, immune enhancer and antioxidant supplement (Erah et al., 2003; Okochi et al., 2003). It has also gained popularity as a remedy for ensuring good joint health and relieving rheumatoid arthritis (Erah et al., 2003; Okochi et al., 2003). A recent survey carried out in Lagos, Nigeria, listed JB as a remedy used by the populace for the treatment of convulsive seizures in children (Oshikoya, 2008). However, no experimental studies have been carried out to confirm the efficacy of JB, as an anticonvulsant agent. Thus, the present study was designed to investigate the effect of JB on chemoconvulsants-induced seizure in mice.

2. Methods

2.1. Experimental Animals

Male albino Swiss mice (20-24 g) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature with 12:12 h light-dark cycle. They had free access to commercial food pellets and water ad libitum. They

were acclimatized for at least one week before use for all experiments. The study was carried out in accordance with the ethical guidelines of the University of Ibadan for the care and use of laboratory animals for experimental studies.

2.2. Drugs and Treatment

Diazepam (Sigma, USA), Jobelyln® (Health Forever Products Ltd, Lagos, Nigeria), picrotoxin (Sigma-Aldrich, St. Louis, USA), strychnine (Sigma, USA) and pentylenetetrazole (Sigma-Aldrich, St. Louis, USA) were used in the current investigation. All drugs were dissolved in distilled water immediately before administration. The doses of 5, 10 and 20 mg/kg of JB used in the study were selected based on the results obtained from preliminary investigations.

2.3. Experimental Procedures

2.3.1. Effect of Jobelyn® on Picrotoxin-Induced Convulsion

Picrotoxin-induced convulsion test was carried out according to the method previously described (Das et al., 2010). Mice were randomly distributed into treatment groups (6 per group) and were given JB (5, 10, 20 mg/kg, p.o.), diazepam (2 mg/kg, p.o.) or distilled water (10 ml/kg; p.o.), 30 min prior to the administration of picrotoxin (6 mg/kg, i.p.). Animals were immediately placed individually in a transparent observation chamber and observed for the expression of convulsions for 30 min after picrotoxin injection.

2.3.2. Effect of Jobelyn® on strychnine-Induced Convulsion

The activity of JB against seizures was also assessed on strychnine-induced convulsions as described earlier (McAllister, 1992). Mice (5 per group) were given JB (5, 10 and 20 mg/kg, p.o.), diazepam (2 mg/kg; p.o.) or distilled water (10 ml/kg, p.o.), 30 min before strychnine (2 mg/kg, i.p.) injection. The time required for the expression of convulsive seizures was assessed by an observer as described before.

2.3.3. Effect of Jobelyn® on Pentylenetetrazole-Induced Convulsion

Pentylenetetrazole-induced convulsion test was employed to further evaluate the anti-convulsant activity of JB as previously described (Loscher et al., 1991). Mice (5 per group) received JB (5, 10 and 20 mg/kg, p.o.), diazepam (2 mg/kg; p.o.) or distilled water (10 ml/

Table 1. Effect of Jobelyn® on picrotoxin-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	6.60±0.24	100	100
JB (5 mg/kg)	6.80±0.37	100	100
JB (10 mg/kg)	7.00±0.44	100	100
JB (20 mg/kg)	9.60±0.93*	100	100
DZ (2 mg/kg)	12.80±0.86*	100	100

Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

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Table 2. Effect of Jobelyn® on strychnine-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	2.40±0.25	100	100
JB (5 mg/kg)	3.70±0.37	100	100
JB (10 mg/kg)	2.40±0.51	100	100
JB (20 mg/kg)	3.60±0.40	100	100
DZ (2 mg/kg)	3.20±0.58	100	100

Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

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Table 3. Effect of Jobelyn® on pentylenetetrazole-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	1.60±0.25	100	100
JB (5 mg/kg)	3.00±0.32*	100	100
JB (10 mg/kg)	3.40±0.51	100	40
JB (20 mg/kg)	2.80±0.20*	100	60
DZ (2 mg/kg)	-	0	0

Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

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kg, p.o.), 30 min before pentylenetetrazole (85 mg/kg, i.p.) injection. The animals were immediately placed individually in a transparent observation chamber and observed for a period of 30 min for the onset of convulsions after pentylenetetrazole injection.

2.3.4. Statistical Analysis

The data were expressed as mean ± S.E.M. The data were analyzed with Graph Pad Prism Software version

4.03. Statistical analysis of data was done by One-way ANOVA, followed by Dunnett's post-hoc test. A level of p < 0.05 was considered as statistically significant.

3. Results

The effects of JB (5-20 mg/kg; p.o.) on convulsions induced by picrotoxin (6 mg/kg, i.p.), strychnine (2 mg/kg, i.p) and pentylenetetrazole (85 mg/kg, i.p) in mice are shown in Tables 1, 2 and 3, respectively. As shown

in the Tables, JB (5-20 mg/kg, p.o) did not provide any protection against convulsions induced by the chemoconvulsant agents in mice. However, JB (5, 10, 20 mg/kg, p.o.) significantly delayed the onset of pentylene-tetrazole- induced seizures (85 mg/kg, i.p). In contrast, while diazepam (2 mg/kg, p.o.) prevented the convulsive seizures produced by pentylene-tetrazole (85 mg/kg, i.p), it was ineffective against picrotoxin- (6 mg/kg, i.p.) or strychnine (2 mg/kg, i.p.)-induced seizure in mice (Tables 1-3).

4. Discussion

The results of this study showed that JB did not provide any protection against the development of seizure episodes induced by pentylene-tetrazole, strychnine or picrotoxin in mice. However, it delayed the onset of seizures and also reduced the mortality rate in pentylene-tetrazole-treated animals. These chemoconvulsants are widely used to induce convulsions in experimental animals (McNamara, 1999). They also served as useful animal models for the development of potential anticonvulsant drugs as well as in exploring the underlying mechanism(s) for their actions (McNamara, 1999). Convulsions may ensue from either a decreased inhibitory synaptic or enhanced excitatory synaptic neurotransmission (McNamara, 1994). The most common type of seizure is febrile convulsions, which occurs frequently in children (Gokhan & Ercument, 2010). It is an acute symptomatic convulsion triggered by fever without the presence of any form of known CNS abnormalities. Febrile seizure is ranked among prevalent neurological disorders in the pediatric age (Gokhan & Ercument, 2010). Synaptic neurotransmitters implicated in convulsions are mostly glutamate, glycine and gamma-aminobutyric acid (GABA). These transmitter substances are linked to ion channels which regulate the rate of neuronal excitation (McNamara, 1999).

According to Kendall et al., 1981, the anticonvulsant activity of a novel compound is measured only by its ability to prevent convulsions but also to delay the onset of seizures or to reduce death rate and/or to decrease the frequency of the episodes. Thus, the ability of JB to delay the onset of seizures, and to reduce death rate in pentylene-tetrazole-treated animals suggests its possible effectiveness to control convulsive episodes. Previous studies have shown that compounds that could delay the onset of convulsions or reduce the frequency of the episodes in experimental animals are capable of halting the spread of seizures in epileptic brains (Corda et al., 1982). However, more detailed studies are required to

verify how JB may be acting to arrest convulsive seizures in epileptic brains.

The differential effects of diazepam on convulsions induced by picrotoxin, strychnine or pentylene-tetrazole, may be related to the dissimilarity in their mode of action. Pentylene-tetrazole produced convulsions by blocking GABAA receptors thereby impairing GABA-mediated inhibitory neurotransmission (Gnyther, 1986; Oni et al., 2009). On the other hand, picrotoxin acts through blockade of chloride ion channel which is known to be resistant to most anticonvulsant agents (Corda et al., 1982; Zetler, 1981). However, strychnine acts by antagonizing glycine receptors, thereby increasing the rate of neuronal excitability (Sayin et al., 1993). Thus, the effectiveness of diazepam against pentylene-tetrazole-induced seizures may be related to its well known action of potentiation of GABA-mediated inhibitory neurotransmission (De Sarro, et al., 1999). However, its failures to prevent seizures induced by picrotoxin may be related to the inability of diazepam to reopen the closed chloride ion gates (Corda et al., 1982; Zetler, 1981).

Jobelyn[®] has been shown to possess various bioactive elements including the well- characterized compounds, such as apigenin, luteolin and naringenin (Erah et al., 2003; Okochi et al., 2003; Yi et al., 2010; Awika & Rooney, 2004). These compounds have been reported to show antidepressant, anti-amnesic, anti-inflammatory and membrane stabilizing properties (Yi et al., 2010; Awika & Rooney, 2004; Heo et al., 2004; Weichiel et al., 1999). However, the role of these compounds in convulsive disorders remains to be confirmed experimentally.

5. Conclusion

The data obtained from this study suggest that JB delayed the onset of seizures produced by pentylene-tetrazole supporting the hypothesis of its relevance in controlling the spread of seizure in epileptic brains.

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