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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2010, **7(2),** 612-616

Anti Epileptic Activity of *Morinda Citrifolia* Linn Fruit Extract

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Received 7 September 2009; Accepted 1 November 2009

Abstract: Fruit extract of *Morinda citrifolia* (Noni), a medicinal plant used in many neuro protective ayurvedic preparations was evaluated for its protective effect against seizures induced by Maximal Electro Shock (MES) method in rats. A daily dose of 200 and 400 mg/kg of the extract was administered to the animals for 15 days, after which seizures were induced by maximum electro shock method and the duration of various phases of epileptic attacks were recorded and compared with the control animals. A significant ($P<0.01$ and $P<0.001$) reduction in the time taken for righting reflex (recovery) was noted in the experimental animals. The levels of biogenic amines such as dopamine, serotonin and nor-adrenaline in the forebrain region were also estimated and a significant level of restoration was observed in the extract treated animals. Significant results were observed in the estimated parameters thereby justifying the use of this medicinal plant in the treatment of epilepsy.

Keywords: *Morinda citrifolia*; Anti epileptic activity; Biogenic amines; Maximal Electro Shock.

Introduction

Traditional medicinal practices have remained as a component of health care system of many societies in spite of the availability of well established alternatives[1]. Epilepsy is a condition, which causes seizures to occur. It is one of the most common chronic diseases affecting human beings. According to several publications this can amount to 70% of the people with epilepsies, with a high prevalence of about 0.8% in children below the age of seven years[2]. These observations have led to a shift in focus to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of

people and are not usually as expensive as the more refined orthodox drugs. Besides, these orthodox drugs possess many side effects, contraindications and possible interactions with drugs used simultaneously. The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles.

Morinda citrifolia Linn Rubiaceae known commercially as Noni grows widely throughout the Pacific and is one of the most significant sources of traditional medicines among Pacific island societies. The Noni plant is used in combinations for herbal remedies. The fruit juice is in high demand in medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, gastric ulcers, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problem, and drug addiction[3].

A number of major components has been identified in the Noni plant such as octoanoic acid, potassium, vitamin c, scopoletin, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, rubiadia-1-methyl ether, antraquinone glycosides) β-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, alizarin, amino acids, acubin, *L*-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin and putative proxeronine[4]. Antitumor activity expressed in enhanced survival of tumor-bearing mice has been demonstrated after treating with juice extracts[5,6]. Aqueous extracts of roots were shown to have an analgesic effect on mice without any sign of toxicity and a sedative effect at high doses[7]. In our previous study we have reported the anti ulcer activity of the *M.citrifolia* fruit extract[8].

The present study has been conducted to evaluate the antiepileptic activity of ethyl acetate extract of *Morinda citrifolia* fruit since this extract has potent anti oxidant activity and neuro protective properties as previously reported.

Experimental

Morinda citrifolia fruits were collected from Abhirami Botanicals, Tuticorin, Tamilnadu. The plant was identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy), Central Research Institute for Siddha, Chennai, India. A voucher specimen was deposited in our laboratory for future reference.

Preparation of extract

The samples were washed with running tap water and separated before being chopped into pieces. They were oven-dried at 45 ^{o}C for 2 days and ground to powder. The ground powder was extracted with methanol in a water bath at room temperature for 24 h. The solvent was then removed by filtration and fresh solvent was then added to the plant material. The extraction process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give a dark green viscous mass. This methanol crude extract was further extracted with ethyl acetate and water, and then separated using separating funnels. These ethyl acetate-soluble fractions were later evaporated and afforded the ethyl acetate extract 9. The extract was stored at 0-4 ^{o}C. The percentage yield was 16%w/w. This extract was used for animal administration.

Healthy adult wistar albino rats between 2 and 3 months of age and weighing about 200-250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; 27±1 °C; 60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water ad libitum. Conduct of the study was approved y the Institutional Animal Ethical Committee of CLBMCP, Chennai, India (Approval No: IAEC/XII/06/CLBMCP/2007-2008 dated 20-04-2007).

Drugs and chemicals

Serotonin, dopamine and nor adrenaline used in the standard readings for the estimation of bioamines were obtained from Sigma (USA) and other chemicals used were of analytical reagent grade.

Acute toxicity studies

Wistar albino rats of either sex weighing 200-250 g selected by random sampling technique was performed as per OECD-423 guidelines (acute class method)[10]. The animals were fasted overnight, provided only water, after which the *M.citrifolia* fruit extract was administered to the respective groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups, observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h.

Estimation of duration of epileptic seizures

The animals were divided into four groups (n=6) and group I animals served as control receiving 1 mL of 5% CMC p.o, group II served as drug control receiving phenytoin 20 mg/kg, p.o and group III and IV animals were administered with the *M.citrifolia* fruit extract at doses of 200 and 400 mg/kg, p.o for 15 days respectively. On the 15th day, seizures were induced to all the groups of animals using electro convulso meter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electro shock (MES) seizures for 0.2 second. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities[11]. The observed duration of various phases of epilepsy was tabulated.

Estimation of biogenic amines

The animals were divided into five groups (n=6) and Group I animals served as control for reference standards, Group II animals served as negative control receiving 1 mL of 5% CMC p.o, group III served as drug control receiving phenytoin 20 mg/kg p.o, group IV animals were administered with the *M.citrifolia* fruit extract at a dose of 200 mg/kg p.o and group V animals received *M.citrifolia* fruit extract at a dose of 400 mg/kg p.o for 15 days. On the 15th day, seizures were induced to all the groups except group I animals using electro convulso meter and biogenic amines in the fore brain of the rat were estimated[12]. The rats were sacrificed by cervical dislocation, since sacrificing by over dose of anesthesia may alter the brain monoamine levels[13]. After sacrificing, the brain was rapidly removed and the fore brain was dissected on a cooled microtome at 20 ^{o}C. The fore brain region was weighed and fore brain of two rats of the same group were pooled and homogenized with 6 mL of cold acidified butanol. Each homogenate pool served as a tissue sample for the respective groups. Internal standards were prepared by the addition of known amounts of mixed standards, (500 µg each of noradrenaline, dopamine and serotonin). The readings were limited to the neither excitation maxima 395-485 nm for noradrenaline, 330-375 nm for dopamine and 360- 470 nm for serotonin. The results were expressed as ng/g of wet brain tissue[14].

Results and Discussion

Effect on MES induced Seizures

The *M.Citrifolia* fruit extract exhibited a dose dependent significant ($P<0.01$ and $P<0.001$) reduction in various phases of epileptic seizure on comparison with the reference standard

phenytoin 20 mg/kg, p.o. There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups (Table 1).

Table 1. Effect of *Morinda citrifolia* fruit extract on MES induced convulsions in rats.

Group	Drug	Flexion, sec	Extension, sec	Clonus, sec	Stupor, sec	Recovery, sec
I	Control	5.2±0.86	13.6±0.89	13.8±1.65	5.86±1.08	186.6
II	Phenytoin 20 mg/kg	3.6±0.58a***	0	8.8±1.69a***	1.18±0.68a***	178.2
III	*M.Citrifolia,* 200 mg/kg	3.36±0.36b***	1.36±0.36b***	5.35±0.35b***	25±1.76b**	136.67
IV	*M.Citrifolia,* 400 mg/kg	2.36±0.26 b***	1.18±0.18 b***	4.86±0.98 b***	19±0.59 b**	115.60

Values represent mean of six observations. Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test. *$P< 0.05$, **$P< 0.01$, ***$P < 0.001$.

Effect on biogenic amine estimation

A significant $P < 0.001$ increase in the dopamine, serotonin and noradrenalin level was noted in the fore brain region for extract treated animals (Table 2).

Table 2. Effect of *Morinda citrifolia* on levels of biogenic amines in forebrain of epilepsy induced rat.

Group	Drug	Serotonin, ng/g of wet tissue	Dopamine, ng/g of wet tissue	Noradrenaline, ng/g of wet tissue
I	Control	168.58±1.72	398.26±2.86	96.6±1.36
II	MES	64.06±0.68a***	126.5±0.22a***	34.89±0.57a***
III	Phenytoin, 20 mg/kg	86.75±0.96b***	256.86±2.28b***	55.46±1.24b***
IV	*M.Citrifolia,*200 mg/kg	93.82±0.86c***	302.46±0.86c***	72.80±1.12c***
V	*M.Citrifolia,* 400 mg/kg	106.24±0.86c***	324.68±0.96c***	68.36±1.86c***

Values represent mean of six observations. MES = Maximal Electro Shock Induced Group. Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test. *$P< 0.05$, **$P< 0.01$, ***$P < 0.001$.

A significant reduction in the time required for the recovery (righting reflex) was observed in this study (Table 1), which proves that *M.citrifolia* fruit extract was providing a beneficial effect in controlling MES induced seizures. It was not surprising that the administration of *M.citrifolia* fruit extract significantly increased the brain levels of serotonin, dopamine and noradrenaline, which could be attributed to the significant protection offered against MES induced seizures (Table 1). The increase in the brain monoamine level by inhibiting the monoamine oxidase (MAO), an enzyme responsible for destruction of biogenic amines tends to raise the seizure threshold[15]. Serotonin (5-Hydroxy tryptamine) is an inhibitory neurotransmitter involved in the regulation of mood, sleep, anxiety, arousal and aggression.

Serotonin agonists, precursors and neuronal uptake inhibitors are reported to enhance narcoleptic catalepsy[16]. The increase in the serotonergic transmission raises the threshold of pentylenetetrazole (PTZ) induced seizures in many animal test systems, thereby protecting against PTZ induced convulsions[15]. Dopamine activation seems to be crucial with respect to a lasting internal encoding of motor skills. Dopamine is also believed to provide a teaching signal to parts of brain responsible for acquiring new behavior. In insects, a similar effect has been demonstrated with respect to octopamine, a chemical relative of dopamine[17]. These effects are mediated by dopminergic receptors situated in several parts of brain including substantia nigra.

Noradrenaline has also a role to play in the control of seizures, but less significantly when compared with other biogenic amines, as it is mainly concerned with blood pressure regulation. It has a potential for biphasic effect of glutamate in the cerebellum and would inhibit glutamate release at low concentrations[18]. Over activation of glutamate receptors may lead to delayed neuro degeneration as a result of increased influx of calcium ions into neurons. The well-established drugs like phenytoin, carbamazepine and benzodiazepines exerts their action by inhibiting calcium calmodulin stimulated protein phosphorylation in presynaptic nerve terminal[11]. A low concentration of dopamine in cerebellum also has an inhibitory effect on glutamate[18]. Inhibition of prostaglandin synthesis is reported to increase the brain levels of dopamine and noradrenaline, which also causes an inhibition of seizure activity[19].

Conclusion

In conclusion, we have found that administration of *M.citrifolia* fruit extract for 15 days increased the seizure threshold in MES induced rats and its possible mechanisms may be due to the inhibition of prostaglandin synthesis and monoamine oxidase enzyme. One more possible mechanism involved in the antiepileptic effect of PHE may be by the decreased influx of calcium ions. The exact mechanism of action of each individual principle remains to be studied in our laboratory.

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