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EVALUATION OF ANTICONVULSANT AND ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF SYZYGIUM AQUEUM (BRUM.F)

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ABSTRACT

Syzygium aqueum (S.aqueum), a species in the Myrtaceae family, commonly called the Water apple is native to Malaysia and Indonasia. It is well documented as a medicinal plant and various parts of the tree have been used in traditional medicine, for instance as antibiotic. The plant also possess some anticancer, antioxidant and anti-hyperglycaemic activities. The objective of the present study was to evaluate the anxiolytic and anticonvulsant activity of the methanolic leaves extract of Syzygium aqueum. After acute toxicity test, oral treatment with Methanolic leaves extract of S.aqueum at doses of 125, 250, and 500 mg/kg behavioral models of elevated-plus-maze, open field, pentylenetetrazole, and maximal electroshock induced seizure models were utilized. In open field test, Methanolic extract of S.aqueum (125, 250, and 500 mg/kg) increased the number of rearings and number of square crossed. Likewise, the number of entries and the time spent in open arm were increased in elevated-plus-maze test. Methanolic leaves extract of S.aqueum (125–500 mg/kg) protected the mice against the pentylenetetrazole induced convulsions; it causes significant dose dependent increase in latency of convulsion. Treatment with Methnolic leaves extract of S.aqueum reduced the duration of the tonic hind limb extension induced by electroshock.

KEYWORDS: S.aqueum, Anticonvulsant, Anxiolytic.

1. INTRODUCTION

A seizure (from Latin “sacire”, to take possession of) is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of central nervous system neurons. Epilepsy describes a condition in which a person has recurrent seizures. This definition implies that a person with a single seizure does not necessarily have epilepsy.[1] Epilepsy is the second most common neurological disorder after stroke.[2] About 50 million people worldwide have epilepsy, and nearly two out of every three new cases are discovered in developing countries. Epilepsy is more likely to occur in young children and elderly, however, it can occur at any time.[3]

Epilepsy if untreated can lead to impaired intellectual function or death.[4] It is a significant clinical problem due to inefficiency of current medication to cure seizures and subsequently side effects like drowsiness and cognitive impairment. Despite the massive scale of the problem and much research, epilepsy remains poorly understood. Presently available therapy is symptomatic, i.e. the drugs inhibit seizures, but whether any of these prevent the development of epilepsy (epileptogenesis) is uncertain. Insights that promise to provide molecular targets for both symptomatic and preventive therapies are being researched.[5]

The heterogeneity of the disease and our limited understanding of it makes discovery and development of anti-epileptic difficult.[1] Medicinal plants are in use since ages for neurological disorders. The added advantage of these would include its complementary nature to conventional treatment making them safer, well tolerated, economical and naturally accessible remedy.

Anxiety is a highly prevalent psychological and physiological state characterized by psychomotor tension, sympathetic hyperactivity, and apprehension and vigilance syndromes and affecting one-eighth of the total population of the world and became a very important area of research interest in psychopharmacology.[6] Synthetic anxiolytic drugs such as benzodiazepines (BDZ), diazepam (DZP), and buspirone (BUSP) are considered as the main category of compounds prescribed for treatment of anxiety disorders. Unfortunately, they have several side effects such as tolerance, amnesia, weakness, loss of sexual drive, gastrointestinal effects and changes in body weight, sedation, muscle relaxation, and physical dependence, which lead patients to seek alternative therapies.[7] In an attempt to resolve these issues, interest has increased in alternative plant-related drugs. Several studies have shown that several ethnomedicinal/traditional medicinal plants have been documented for the treatment of central

nervous system (CNS); these ethnomedicinal plants could serve as sources of effective medication that may be more readily accessible and inexpensive and thus would be helpful in improving the present status.[8,9]

Syzygium aqueum is a brush cherry tree in the Myrtaceae family, commonly called water rose apple. It is well documented as a medicinal plant and various parts of the tree have been used in traditional medicine, for instance as an antibiotic. The plant also possess some anticancer, antioxidant and antihyperglycaemic activities.

2. MATERIALS AND METHOD

2.1. Plant collection and Identification

The species for the proposed study that is leaves of Syzygium aqueum has carefully collected from Angadippuram, Malappuram DT, Kerala.

The plant was positively identified by Dr. Prabhukumar.K.M, Senior Scientist and Head, Plant Systematics and Genetic Resourse Divison and CMPR Herbaria, Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal.The plant was authenticated as Syzygium aqueum (Brum.f.) Alston of Myrtaceae family.

2.2. Preparation of Plant extract

The Syzygium aqueum leaves were subjected to shade drying to treat fungus until complete dryness of leaves. Then the dried leaves were powdered by mixer grinder until to get coarse powder. About 250gm of air dried powdered material was taken in 3000ml soxhlet apparatus and extracted with petroleum ether until green colour disappear. At the end of the day the powder was taken out and dried. After drying it was again packed and extracted by using Methanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. The temperature was maintained at 55ºC-65ºC. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of Methanolic extract were noted. The yield was found to be 7% w/w.

2.3. Drugs and Chemicals

Diazepam (Calmpose Inj. Ranbaxy, India), Pentylenetetrazole (Sigma, USA), Methanol extra pure (S.D fine chemicals, Mumbai).

2.4. Animals

Albino mice of either sex 20-30 gm of body weight obtain from Animal House, Department of Pharmacology, J.K.K.M.M.R.F. College of Pharmacy, (Proposal No. – JKKMMRFCP/IAEC/2017/007) Namakkal DT, Tamil Nadu. Animals were kept in standard animal house condition. Prior to use, the mice were housed in polypropylene cages in group of six animals under natural light-dark cycle. They were provided with commercial food pallets and tap water *ad libitum*. Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material. All the observations were made at room temperature in a noiseless diffusely illuminated room. The cages were maintained clean and all experiments were conducted between 8 am to 3 pm.

2.5. Acute toxicity study

Acute toxicity of Methanolic leaves extract of Syzygium aqueum was evaluated according to the method described by Organization of Economic Cooperation and Development Guideline 423. The animals were kept fasting for overnight providing only water. The extract was administered orally at a dose of 5 mg/kg (suspended in 0.5% carboxymethyl cellulose (CMC)) initially to separate groups of mice and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only 1 mouse out of 6 animals, then the same dose was repeated with higher doses such as 50, 300, 500, 1000, and 2,000 mg/kg b.w. orally. The control mice were given 10 mL/kg of water. The mice were observed for behavioural changes and mortality within 24 h. After this, daily observations for toxicity and mortality were made up to the 14th day.

2.6. Anxiolytic Test

2.6.1. Elevated plus maze (EPM) test

The EPM test is the most frequently employed model for the assessment of the anxiolytic activity of novel substances.[10] The elevated plus maze apparatus consisted of two perpendicular open arms (50 X 10 cm) and two perpendicular enclosed arms (50 X 10 X 40 cm). The entire maze was constructed of wood and elevated 50 cm above floor. The maze was placed inside a light (25 lx) and sound attenuated room.

The animals were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic Extract of Syzygium aqueum at doses of 125, 250, and 500 mg/kg, body weight. Thirty minutes later, the rat was placed in the center platform of the maze facing the enclosed arm and was observed for 10 min. The parameters assessed were the time spent in open and enclosed arms and numbers of open and enclosed arms entries. All tests were taped by using a video camera and every precaution was taken to ensure that no external stimuli could evoke anxiety in the mice. After each test, the maze was carefully cleaned up with a wet tissue paper (70% ethanol solution) to eliminate the interference of the olfactory cues on the next rat.[11]

2.6.2. Open field test

The study was conducted according to method previously described by Brown et al with some modifications. The apparatus was made up of plywood measuring 72 cm X 72 cm X 36 cm. One of the walls was made of transparent Perspex glass to ensure that the mouse under investigation is visible to the observer. The

floor, made of cardboard, was divided into 16 equal squares (18 cm X 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas. The animals were divided into five groups; each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Syzygium aqueum at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, each mouse was placed individually at the corner of the arena and its behavior monitored for 5 min. The number of rearings and number of square crossed by each mouse was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

2.7. Anticonvulsant test

2.7.1. Pentylenetetrazole Induced Convulsions

Pentylenetetrazole (PTZ) induced convulsions test was performed to evaluate anticonvulsant property of drugs.[12] Thirty male mice were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Syzygium aqueum at doses of 125, 250, and 500 mg/kg, body weight. Thirty minutes later, convulsions were induced by the intraperitoneal administration of 60 mg/kg BW of PTZ. Following the administration of PTZ, mice were placed in separate transparent plexiglass cages (25 × 15 × 10 cm) and were observed for the occurrence of seizures over a 30 min time period. Latency of convulsions (the time prior to the onset of tonic convulsions), duration of tonic convulsions, and mortality protection (percentage of deaths in 24 h) were recorded.[13]

2.7.2. Maximal Electro Shock (MES) Induced Convulsions

The animals were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Syzygium aqueum at doses of 125, 250, and 500 mg/kg, body weight. Thirty minutes later, convulsions were induced in all the groups of animals using electro convulsiometer. A 60 Hz alternating current of 150 mA for 2 s was delivered through the ear electrodes.[14] The animal was observed for the occurrence of tonic hind limb extension.

2.8. Data analysis

Results of the experiments and observations were expressed as mean ± standard deviation (SD). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by at least one of the following post hoc tests: Dunnett's multiple comparison tests $P< 0.05$ where level of significance was considered for each test. The data is presented as mean ± S.D.

3. RESULTS AND DISCUSSION

3.1Acute oral toxicity studies

The acute oral toxicity of the Methanolic extract of Syzygium aqueum was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that LD50>2000mg/kg for the extract. Hence, the biological dose was fixed at 125, 250mg and 500mg/kg body weight.

3.2. Anxiolytic activity

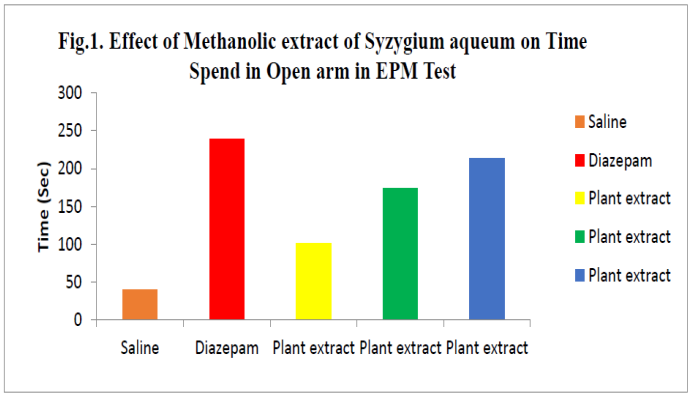
3.2.1. Elevated plus maze: Administration of diazepam (5mg/kg) significantly increases number of open arm entries, time spent in open arms and the number of rearings in open arm. They showed a reduction in the time spent in closed arm. Plant extracts treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm as shown in the table 1.

3.2.2. Open field test: There was significant anxiolytic activity observed with diazepam, plant extracts when compared to control. In the open field test, plant extract showed significant increase in number of rearings, number of squares crossed and number of assisted rearings during 5 min intervals of test as compared with control as show in table 2.

Table. 1: Effect of Methanolic extract of Syzygium aqueum on Elevated plus maze in mice.

Group	Treatment	Dose	Time spent in open arm (s)	Entries in open arm
I	Saline	10ml/Kg	40.25±4.41	3.98±0.52
II	Diazepam	5mg/kg	239.59±3.52**	12.64±0.47**
III	Plant extract	125mg/kg	100.83±3.97	6.48±0.39
IV	Plant extract	250mg/kg	173.81±4.32*	6.53±0.42*
V	Plant extract	500mg/kg	213.92±4.80**	10.32±0.21**

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001significantly different compared to normal control and diazepam.



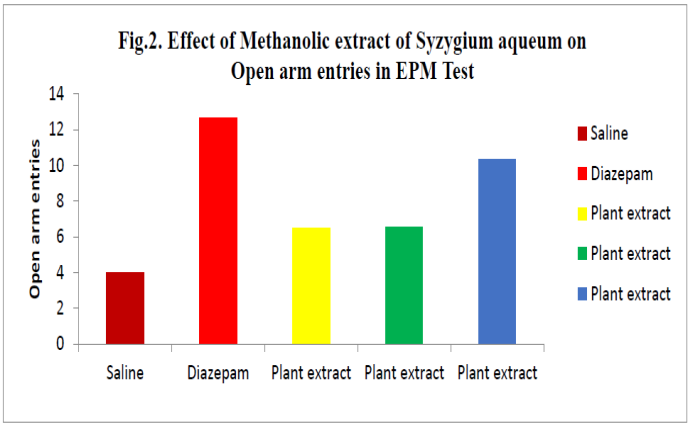
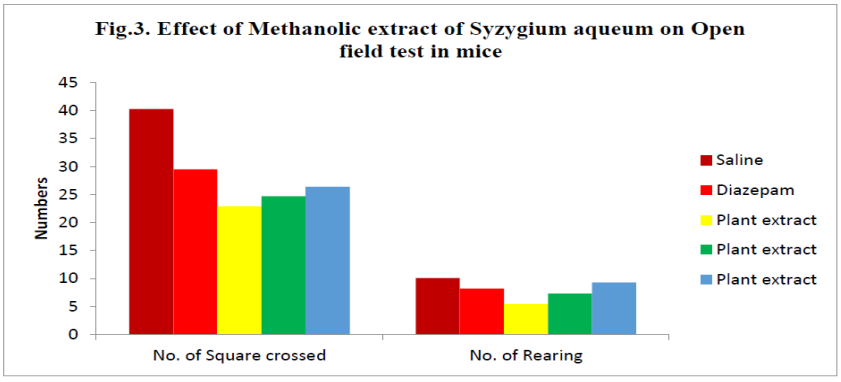


Table.2. Effect of Methanolic extract of Syzygium aqueum on Open field test in mice.

Group	Treatment	Dose	Number of square crossed	Number of rearing
I	Saline	10ml/kg	40.3±2.1	10.1±1.4
II	Diazepam	5mg/kg	29.5±3.6**	8.2±1.8**
III	Plant extract	125mg/kg	22.9±2.4	5.4±2.3
IV	Plant extract	250mg/kg	24.7±3.2*	7.3±3.5*
V	Plant extract	500mg/kg	26.4±2.8**	9.3±1.2**

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam.



3.3. Anticonvulsant activity

3.3.1. PTZ Induced Convulsion: Pentylenetetrazole produced tonic seizures in the entire animals used. A dose of 125 mg/kg of Methanolic extract of leaves of Syzygium aqueum protected 33.33% of the animals against seizures and did not affect the onset (latency) of seizures to any significant extent. Methanolic extract of leaves of Syzygium aqueum at the dose of 250 and 500

mg/kg protected 50.0% and 100% of the mice against seizures and increased the latency of the seizures (Table 3).

3.3.2. Maximal Electro Shock Model: Maximal electroshock produced hind limb tonic extension (HLTE) in all the animals. The vehicle treated mice showed tonic hind limb extension for duration of12.88 ± 0.35 s. Administration of Methanolic extraction of leaves of Syzygium aqueum (125–500 mg/kg) showed a dose dependent increase in the delay of the onset time of seizures induced by maximal electroshock induced convulsion and also decreased duration of tonic hind limb extension (Table 4).

Table.3. Effect of Methanolic extract of leaves of Syzygium aqueum on PTZ induzed convulsions in mice.

Group	Treatment	Latency of Tonic convulsion (s)	Duration of Tonic convulsions (s)	Mortality (% death)	% Protection
I	Control	100.20±3.34	446.10±5.19	6/6(100)	0.0
II	Diazepam (5mg/kg)	478.34±6.07**	126.69±1.93**	0/6(0.0)	100
III	Plant extract (125mg/kg)	141.43±1.98	216.29±1.23	4/6(66.66)	33.33
IV	Plant extract (250mg/kg)	298.16±4.45*	189.19±1.72*	3/6(50.00)	50.0
V	Plant extract (500mg/kg)	416.42±6.14**	137.11±2.61**	0/6(0.0)	100

The data represents the mean S.D ± (n=6) *p<0.1, **p<0.001significantly different compared to normal control and diazepam.

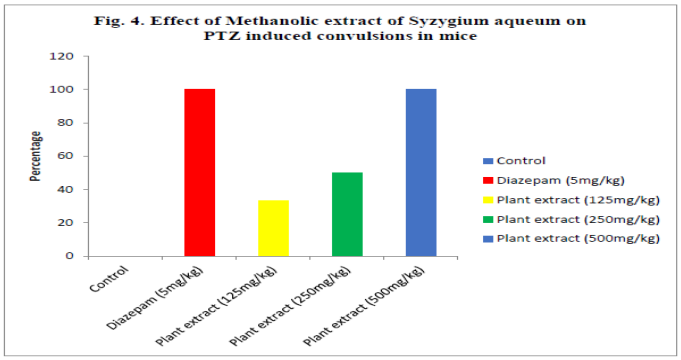
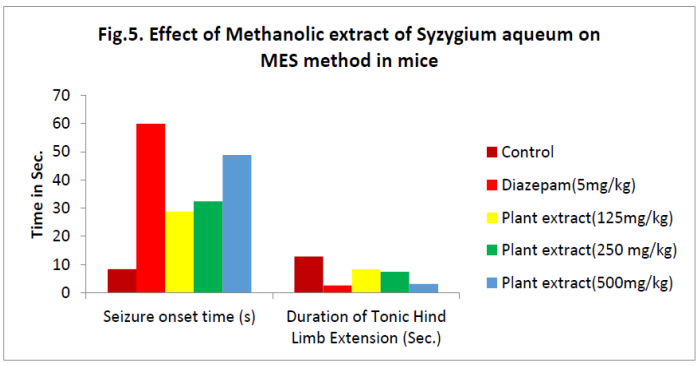


Table. 4: Effect of Methanolic extract of Syzygium aqueum on Tonic seizures induced by MES method in mice.

Group	Treatment	Seizure onset time (s)	Duration of Tonic Hind Limb Extension (Sec.)
I	Control	8.38±1.88	12.88±0.35
II	Diazepam(5mg/kg)	59.88±1.35**	2.63±1.72**
III	Plant extract(125mg/kg)	28.81±1.10	8.28±1.19
IV	Plant extract(250 mg/kg)	32.43±1.44*	7.44±1.01*
V	Plant extract(500mg/kg)	48.84±1.25**	3.21±1.25**

The data represent the mean ±S.D (n=6) *p<0.05, **p<0.001 significantly different compared to normal control and diazepam.



4. CONCLUSION

Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication since the earliest times known to man. Several ethnomedicinal plants have been found to possess neurobehavioral profile and serve as alternative to modern medicine. Biological evaluation and scientific validation of the ethnomedicinal plants are the need of the hour.[15,16] The present study was proposed to assess anxiolytic, and anticonvulsant effects of methanolic extract of leaves of Syzygium aqueum.

The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight.

Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopamanergic system have also been shown to play a role in anxiety. BZA have been extensively, used for the last 40 years to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favorable side effect profiles. Medicinal plants are a good source to find new remedies for these disorders. Despite the wide spread traditional use of Syzygium aqueum for treating various disorders there are no reports of scientific evaluation of its anxiolytic and anticonvulsant activity. The present work demonstrates that the Syzygium aqueum leaf extract had anxiolytic activity in mice by Elevated Plus Maze and Open field models.

Elevated Plus Maze is used to evaluate psychomotor performance and emotional aspects of rodents. Results showed that plant extracts treated mice exhibited significant increase in the number of open arm entries but decreases in time spent in closed arm, which reflects plants anxiolytic property.

The open field test is used to evaluate the animal emotional state. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Mice treated with extract showed increase in number of rearings and time spent in the center.

The results of the present laboratory animal study indicate that Methanolic extract of Syzygium aqueum leaf extract possesses anticonvulsant activity. The present study demonstrated the anticonvulsant effects of the methanolic extract of Syzygium aqueum in both chemically and electrically induced seizures in mice. The extract exhibited dose dependent protection in the MES and PTZ induced convulsions. Nevertheless, in unprotected animals, the extract significantly increased seizure latency and reduced seizure duration compared with the control group in all two models at all tested doses. The effect of most of antiepileptic agents is to enhance the response to GABA by facilitating the opening of GABA-activated chloride channels. GABA receptors were involved in epilepsy and their direct activation would have an antiepileptic effect.

The anticonvulsant, anxiolytic, and sedative effects of benzodiazepines like diazepam are mostly attributed to enhance the action of gamma-aminobutyric acid (GABAA).[17] Actually, benzodiazepines bind to the gamma subunit of the GABAA receptor, due to which a structural modification of the receptor results in an increase in GABAA receptor activity. Benzodiazepines do not substitute for GABA, which bind at the alpha subunit, but increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential.[18,19] According to some researchers, the anxiolytic action of benzodiazepines may be due to the direct activation of glycine synapses in the brain.[18,20] This may explain the mechanism of action of the tested extract as well, because it is clear from the results that the effect of the extract was similar to diazepam.

Previous phytochemicals reported in the literature, various Flavonoids, glycosides, Alkaloids and triterpenoids, isolated from Syzygium aquem would be the effective constituents for their anxiolytic and anticonvulsant effect.

In conclusion, Methanolic extract of Syzygium aqueum possesses anxiolytic and anticonvulsant effects and these findings collaborate with the ethnomedicinal uses of this plant. The isolation of active chemicals from this plant might serve as lead compounds for the synthesis of drugs which could be used in the management of these nervous disorders.

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