Anticonvulsant and neuroprotective effects of the *Acacia tortilis* growing in KSA

Waheeb Dakhelallah Mohammad Alharbi and Aisha Azmat
Department of Physiology, Faculty of Medicine Umm-Al-Qura University, Makkah, Saudi Arabia

Abstract: In different region of Saudi Arabia *Acacia tortilis* (Fabaceae) is present but still the medicinal properties of *Acacia tortilis* have not been studied. However, in Zimbabwe different species of *Acacia* are already used for the treatment of convulsions and dizziness. In the present study, the anticonvulsant and neuroprotective effects of the *Acacia tortilis*, were evaluated by using different paradigms. For extraction, the leaves of acacia were blended with distilled water at 40°C and filtered. Two different doses of the extracts (400 and 800mg/kg) were administered in the mice once orally (p.o.) and after 30 min occurrence of seizures (strychnine at the dose of 1mg/kg, i.m.) were monitored. In the present work, acute toxicity and neurotoxicity of the extracts were also assessed by inducing hypoxic stress. The *Acacia tortilis* leaves AAq (400 and 800 mg/kg) produced a dose dependent increase in time of onset of seizures (197.8±32.4 and 338.2±40.6 respectively) when compared with its respective control (184.0±13.8sec). The anticonvulsant effect after administration of AAq (800mg/kg: 338.2±40.6 sec) was more pronounced than diazepam (290.6±1.38 sec). The high dose (800mg/kg) of AAq administered orally prolonged the onset of convulsion and latencies for death following hypoxic stress. The present study suggested that *Acacia* have anticonvulsant property and may probably be affecting the inhibitory mechanism of glycine. It is also concluded that chemical constituent of acacia might act on BZD or 5-HT_{1A} receptor and decrease the oxidative brain membrane damage process induced by psychological/hypoxic stress. Further experiments will be required to identify the active molecules (s) and their mechanism (s) of action.

Keywords: *Acacia tortilis*, strychnine, hypoxic stress, neuro-degeneration, DMT.

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. Incidence of epilepsy in elderly is greater than in teens and children (Brodie *et al*., 2009). Holmes *et al*., 2008 suggest that by the age of 20 epilepsy affects 1% population and 3% people by age 75. Another study revealed that approximately 63,400 or 1 in 220 children and young people aged 18 years and under are suffering with epilepsy, while the incidence of epilepsy is higher in people aged 65 and over that is approximately 154,000 or 1 in 67 (Joint Epilepsy Council 2011). Different drugs are used to alleviate the symptoms of epilepsy. A previous report stated that 25% drugs are unable to alleviate the symptoms of seizures (Patil & Saini, 2012). Furthermore, almost all available drugs may produce side effects. Because of low efficacy and increase side effect of antiepileptic drug, it is important to search for new drugs having naturally-occurring compounds. Different plants and their active constituents are used as medicine for the cure of diseases in herbal medicine but these plants mostly, have not been explored scientifically. *Acacia tortilis* present in different dry areas of Africa and the Middle East. It is used as food, fuel (Wicken *et al*., 1995), and as a local medicinal plant. Phytochemical screening of *Acacia* reveals the presence of two pharmacologically active compounds isolated from the bark. These compounds are used for the treatment of asthma and in wounds healing. The seeds are used to treat diarrhea (Wickens and Pennacchio, 2002). However, in Zimbabwe different species of *Acacia* are already used for the treatment of convulsions and dizziness (Stafford *et al*., 2008).

Keeping these views in mind it is important to conduct scientific analysis for the physiological, pharmacological and biochemical effects of *Acacia tortilis* present in Makkah. The present study, therefore, aims to provide a preliminary interpretation of the anticonvulsant and neuroprotective activity of *Acacia tortilis* growing in Makkah because it has not yet been explored.

MATERIALS AND METHODS

Collection and extraction of Acacia tortilis

The leaves of *Acacia tortilis* were collected from Makkah region, KSA in October 2011. It was identified and authenticated by Dr. Kadry Abdel Khalik, working in the Department of Plant Taxonomy, UQU, Makkah, Saudi Arabia.

Experimental animals

56 albino Mice (both male and female weighing between 20-25 g) were purchased from King Abdul Aziz University Jeddah and housed in an air-conditioned room at 23±1 C at the College of Medicine, UQU for seven days prior to experimentation. They were feed (rodent

**Corresponding author:* e-mail: aishaazmatkhan@hotmail.com

diet) and allowed to drink tap water, *ad libitum* with 12h dark and light cycle. 36 mice were used for anticonvulsant and Hypoxic stress induced neuro-toxicological studies for that each experimental group has six animals. While 20 mice were used for single dose toxicity had 10 mice in each group. For each experiment fresh mice were used.

The experimental procedures were performed according to Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2010). All experimental Procedure was approved by review board of departmental research committee.

Preparation of extract

50gm of *Acacia* Leaves were washed and rinsed with distilled water. To prepare the water extract, the leaves were grinded in blender with 500ml of distilled water. This mixture was kept on shaking water bath (40°C: half an hour) for mixing and then filtered. This filtrate (100mg/ml) AAq was used for experiments as described earlier by Alharbi and Azmat (2010). Two different doses (400 and 800mg/kg) of AAq were used in present study orally p.o.

Anticonvulsant activity

Mice of either gender were divided randomly in to 4 groups of 6 animals each.

Group I Control mice received normal saline (10ml/kg, p.o)

Group II Positive control group of mice received Diazepam (3mg/kg, i.p)

Group III & Group IV received 400 or 800mg/kg, AAq p.o. respectively.

After 30min, all animals were received intramuscular injection of strychnine (1mg/kg) and onset and duration of convulsions were recorded (Adeyemi *et al*., 2010).

Hypoxic stress induced neurotoxicity in mice

Hypoxia was produced in mice by putting them individually in a 300ml tightly closed glass container at 25^{o}C temperature. The onset of convulsion and latency for death ware recorded. Mice received single dose of AAq (800mg/kg, p.o.) as described by Vyawahare and Ambikar (2010).

Toxicological studies in mice

For toxicological studies mice were divided in to two groups (20-25g) containing ten animals in each group. Group I (5 males and 5 females), was treated with AAq (800mg/kg), while Group II (5 males and 5 females) as control received distilled water only. Any sign of toxicity and mortality was noted up to 24h in each group.

STATISTICAL ANALYSIS

Results are presented as Average ± S.E.M (Number of animals). Statistical analysis of obtained data was done by using one way analysis of variance followed by Tukey's multiple comparison tests. $P<0.05$ was considered as level of significance for each test.

RESULTS

Strychnine induced seizure test

In strychnine-induced seizures model, the anticonvulsive effect of extract appears to be dose dependent as shown in table 1. AAq (800mg/kg) and Diazepam delayed the onset of strychnine-induced seizures (table 1) significantly. The onset of convulsion and time of living increase (control; 217.0±31.2: AAq 344.8±21.3sec) significantly ($p<0.005$) after the administration of AAq (800mg/kg) and diazepam (control; 217.0±31.2: Diazepam 420.4±8.4sec) being statistically significant ($P<0.001$), but AAq at the dose of 400mg/kg did not exhibit significant anticonvulsant effect ($P>0.05$). The results of anticonvulsive activity of AAq showed that the anticonvulsive parameters were better controlled at dose of 800mg/kg.

Neurotoxicity induced by Hypoxic stress in mice

AAq (800mg/kg) significantly ($P<0.05$) prolonged the onset of convulsion (control 24.00±1.79: AAq treated 43.00±8.24 sec) and latencies for death (control 31.60 ±0.678: AAq treated 45.40±6.19 sec) following hypoxic stress when compared to its respective control as shown in table 2.

Toxicological studies

Oral administration of single dose of AAq (400 and 800mg/kg) did not show any change in behavior of mice. None of these mice showed any sign of toxicity and behavioral change after first 2 hour of AAq administration. No death was noted in mice.

DISCUSSION

The anticonvulsant activity of AAq at various doses (400 and 800 mg/kg p.o) was studied by the Chemoconvulsant strychnine-induced seizure models. It is suggested that Strychnine increase spinal reflexes (Rasilingam *et al*., 2009) by directly antagonizing the inhibitory reflexes of glycine in spinal cord and brain stem thus causing convulsions. Positive control Diazepam inhibits the strychnine induced seizures. Diazepam is usually associated with many side effects. In developing countries about 75 to 80% of the population use herbal or traditional medicines, for primary health care because of lesser side effects (Riss *et al*., 2008). In the last three decades, a lot of concerted efforts have been channeled into researching the local plants with anticonvulsant therapeutic values. In the present preliminary study AAq inhibit strychnine induced seizures indicates its effect on the glycine receptors in the spinal cord. Further studies are needed for identifying the exact molecular mechanism for the anticonvulsant activity. Literature survey reveals

Table 1: Anticonvulsant activity of Acacia Extract on strychnine induce seizure in mice

	Onset of convulsion mean ± SE mean (n)	Death of animals mean ± SE mean (n)
Control	184.0±13.8 (6)	217.0±31.2 (6)
Reference drug (Diazepam, 3 mg/kg)	290.6±1.38* (6)	420.4±8.4* (6)
Low dose (400mg/kg)	197.8±32.4*** (6)	211.4±24.2*** (6)
High dose (800mg/kg)	338.2±40.6** (6)	344.8±21.3** (6)

* Indicated the significant difference between control and reference drug (P<0.001)
** Indicated the significant difference (P<0.005) between control and High dose of extract (800mg/kg)
***Indicated the significant difference (P<0.007) between low dose (400mg/kg) and High dose of extract (800mg/kg)
(n) Indicated the sample size

Table 2: The effect of the Acacia leaves extract on Hypoxic stress induced neurotoxicity in mice

	Latencies for Death mean ± SE mean (n)	Onset of convulsion mean ± SE mean (n)
Control	31.60±0.678 (6)	24.00±1.79 (6)
High dose (800mg/kg)	45.40± 6.19 * (6)	43.00±8.24 * (6)

* Indicated the significant difference (P<0.05) between control and High dose of extract (800mg/kg)
(n) Indicated the sample size

that Acacia species used to treat convulsions and dizziness in Zimbabwe (Stafford *et al*., 2008). Previously, the anticonvulsant effect of the leaves of *Acacia juliflora*, *Acacia nubica* and aerial parts of Astragalusobtusifolius was evaluated in pentylenetetrazole (PTZ) and maximal electroshock (MES) seizure tests (Sayyah *et al*., 2011) showed anticonvulsant activity. Sayyah *et al*., 2011results were in agreement with the present result. It seems that alkaloids, flavonoids and saponins present in the Acacia extract (Almahy and Nasir, 2011) and the aqueous fraction might be mainly responsible for the observed anticonvulsant activity. In agreement with this suggestion the anticonvulsant activity of AAq is because of the presence of flavonoids (Fernandez *et al*., 2006), saponins (Chindo *et al*., 2009) and alkaloids (Bhutada *et al*., 2010).

Hypoxia cause oxidative damage and neurodegeneration. The incidence of hypoxic stress are increasing and affecting the cognitive behavior. In different case it may result in induction of convulsion and death too. In the present study, AAq decrease the onset of convulsion and increase the time of survival in hypoxic mice as shown in table 2. Scientist suggested the presence of N,N-Dimethyltryptamine (DMT) in the *Acacia* leaf (Rovelli, and Vaughan, 1967) belongs to tryptamine family and derived from the essential amino acid tryptophan (Barker *et al*., 1981). Structurally, DMT is analogous to the neurotransmitter serotonin (5-HT). DMT acts as agonist at 5-HT_2 receptors (Smith *et al*., 1998) and 5-$HT1_a$ receptors (Deliganis *et al*., 1991). DMT also block 5-HT uptake at micromolar concentrations (Cozzi *et al*., 2009). It was concluded that agonist drug with a BZD or 5-HT_{1A} receptor have a neuroprotective effect against hypoxic stress induced by psychological stress (Matsumoto *et al*., 1999). Toxicological studies revealed that single dose of AAq (800mg/kg) also prevented mortality in mice.

CONCLUSION

It is concluded that the AAq has anticonvulsant and neuroprotective actions. Although the current research have shown significant anticonvulsant and neuroprotective profile of Acacia but the current study had few limitations such as small sample size, use of single species of animal and used of aqueous extract only. This heralds the need to conduct further research on different animal species with different ethanolic and methanolic extracts to evaluate the role and exact mechanism of action of Acacia.

ACKNOWLEDGEMENT

Authors are grateful to Miss Moodi Saleh Al-lehibi, Miss Jawaher Abdulaziz Al-harthi, Miss Mashael Mohammad Al-masoudi, Miss Hatoon Abdulghani Al-ahdal, Miss Bashaer Zaki Al-nass and Miss Wafaa Ali Bayunus Faculty of Applied Medical Sciences, Umm-Al-Qura University, Makkah, Saudi Arabia, for providing the research assistance.

REFERENCES

Adeyemi OO, Akindele AJ, Yemitan OK, Aigbe FR and Fagbo FI (2010). Anti-convulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longepedunculata Fresen. J. Ethnopharmacol,*, **130**: 191-195.

Alharbi WDM and Azmat A (2011). Hypoglycemic and hypocholesterolemic effects of *Acacia tortilis* (Fabaceae) growing in Makkah. *Pak. J. Pharmacol.*, **28**: 1-8.

Almahy HA and Nasir OD (2011). Phytochemical and mineral content of the leaves of four Sudanese *Acacia*

species. *J. Stored Prod. Postharvest. Res.*, **2**(11), 221-226.

Barker SA, Monti JA and Christian ST (1981). N,N-dimethyltryptamine: An endogenous hallucinogen. *Int. Rev. Neurobiol.*, **22**: 83-110.

Bhutada P, Mundhada Y, Bansod K, Dixit P, Umathe S and Mundhada D (2010). Anti-convulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy Behav.*, **18**: 207-210.

Brodie MJ, Elder AT and Kwan P (2009). "Epilepsy in later life". *Lancet neurology*, **11**: 1019-1030.

Chindo BA, Anuka JA, McNeil L, Yaro AH, Adamu SS, Amos S, Connelly WK, Lees G and Gamaniel KS (2009). Anticonvulsant properties of saponins from *Ficus platyphylla*stem bark. *Brain Res. Bull.*, **78**: 276-282.

Cozzi NV, Gopalakrishnan A, Anderson LL, Feih JT, Shulgin AT, Daley PF and Ruoho AE (2009). Dimethyltryptamine and other hallucinogenic tryptamines exhibit substrate behavior at the serotonin uptake transporter and the vesicle monoamine transporter. *J. Neural Transmission*, **116**: 1591-1599.

Deliganis AV, Pierce PA and Perouka SJ (1991). Differential interactions of dimethyltryptamine (DMT) with 5-HT_{1A} and 5-HT_2 receptors. *Biochem. Pharmacol.*, **41**: 1739-1744.

Fernandez SP, Wasowski C, Loscalzo LM, Granger RE, Johnston GAR, Paladini AC and Marder M (2006). Central nervous system depressant action of flavonoid glycosides. *Eur. J. Pharmacol.*, **539**: 168-176.

Holmes T, Browne R and Gregory L (2008). Handbook of epilepsy (4^{th} ed.). Lippincott Williams & Wilkins, Philadelphia, p.7.

Joint Epilepsy Council, September (2011). Epilepsy prevalence, incidence and other statistics. Pp.1-13.

Matsumoto K, Yobimoto K, Huong NT, Abdel-Fattah M, Van Hien T and Watanabe H (1999). Psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide systems and its modulation by anxiolytic and anxiogenic drugs in mice. *Brain Res.*, **839**(1): 74-84.

National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research. (2010). Guide for the Care and Use of Laboratory Animals. Prepublication draft. 8^{th} edition. The national academies press washington, D.C. 6, 47.

Patil SM and Saini R (2012). Anti-convulsant activity of methanolic extract of *Jasminum grandflorum* Linn in experimental animals. *RJPBCS*, **3**(2): 43-49.

Rasilingam D, Duraisamy S and Subramanian R (2009). Anticonvulsant activity of bioflavonoid *gossypin*. *Bangladesh J. Pharmacol.*, **4**: 51-54.

Riss J, Cloyd J, Gates J and Collins S (2008). Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. *Acta. Neurol. Scand*, **118**(2): 69-86.

Rovelli B and Vaughan GN (1967). Alkaloids of *Acacia I.* Dimethyltryptamine in *Acacia phlebophylla*. *Aust. J. Chem.*, **20**: 1299-1300.

Sayyah M, Khodaparast A, Yazdi A and Sardari S (2011). Screening of the anti-convulsant activity of some plants from Fabaceae family in experimental seizure models in mice. *DARU.*, **19**: 301-305.

Smith RL, Canton H, Barrett RJ and Sanders-Bush E (1998). Agonist properties of N,N-dimethyltryptamine at serotonin 5-HT2A and 5-HT2C receptors. *Pharmacol. Biochem. Behav.*, **61**(3): 323-330.

Stafford GI, Pedersen ME, Van Staden J and Jäger AK (2008). Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *J. of Ethnopharmacol.*, **119**(3): 513-537.

Vyawahare NS and Ambikar DB (2010). Evaluation of neuropharmacological activity of hydroalcoholic extract of fruits of *Trapa bispinosa* in laboratory animals. *Int. J. Pharm. Pharm. Sci.*, **2**: 32-35.

Wicken GE, Seif-El-Din AG, Guinko S and Ibrahim N (1995). Role of *Acacia* species in rural economy of dry Africa and near east. Food and agriculture organization of the united nation. Rome, Italy: FIAT PANIS, Pp.13-15.

Wickens K and Pennacchio M (2002). A search for novel biologically active compounds in the phyllodes of *Acacia* species. *Conservation Science W. Aust.*, **4**(3): 139-144.