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(RESEARCH ARTICLE)

Sedative and hypnotic effects of the roots of *Asparagus africanus* (Asparagaceae) decoction on white mice (*Mus musculus* Swiss)

Tchinmi Elisabeth [1,*], Ngah Esther [2] and Ngo Bum Elisabeth [1]

[1] *Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon.*
[2] *Department of Food Sciences and Nutrition, National School of Agro-Industrial Sciences, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon.*

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Abstract

Asparagus africanus Lam. (Asparagaceae) is a plant widely used in traditional medicine as an anti-inflammatory, for the treatment of nervous disorders and insomnia. The aim of this work was to study the sedative and hypnotic effects of the roots of *A. africanus* decoction on white mice (*Mus musculus* Swiss). Sleep potentiation tests induced by diazepam and sodium pentobarbital were used. The sleep latency period onset and the sleep duration were recorded. The concentrations of GABA a[nd GABA-T in the brains of mice were also estimated. *A. africanus* significantly decreased the sleep latency period onset and increased the sleep duration induced by diazepam and sodium pentobarbital. Bicuculline, a competitive photosensitive antagonist of the GABAA receptor complex, did not prevent this potentiation. The effect of *A. africanus* on the sleep time was not blocked by flumazenil, a specific antagonist to the benzodiazepine site in the GABAA receptor complex. GABA increased and GABA-T decreased in the animals brain *A. africanus* treated significantly. Therefore the sedative properties of *A. africanus* might be possibly mediated by the activation of GABAergic neurotransmission on inhibitory receptors and by the decrease in the recapture of GABA by inhibiting GABA-T. These properties justified its use against insomnia in traditional medicine.

Keywords: *Asparagus africanus*; Diazepam; Pentobarbital sodium; Sedatives; Mice

1. Introduction

Insomnia is a degenerative disease that affects the central nervous system due to high excitement in the brain [1, 2]. It is defined as a complaint of sleep failure but difficult to obtain, insufficient or non-recoverable [3]. Insomnia is a very common health problem that lowers the quality of life of the individual and has significant social and economic costs [2]. It has a prevalence of 35% worldwide, of which 9 to 10% are chronic cases and 25% are occasional cases [4]. The main causes of insomnia are: anxiety, depression and psychoses which impart excessive stress on the temporal lobes [5]. Patients with epilepsy often report non-restful sleep and daytime sleepiness which affects their life quality and can lead to even more seizures [6].

Insomnia is treated with sedatives such as barbiturates (phenobarbital), benzodiazepines (Diazepam) and new generation sleeping pills (zolpidem and zopiclone) [7]. Unfortunately, these sedatives are indeed responsible for phenomena of dependence, intoxication and amnesia. They aren't indicated in pregnancy and lactation as well as for children [7, 2]; hence the need to look for a substance that is efficient, without undesirable side effects and that can be used by all. Medicinal plant-based pharmacotherapy for neurological and psychiatric illnesses has progressed due to their lower undesirable side effects and better tolerance [8]. Plants have extraordinary therapeutic virtues and about 75% of the african population still relies on plants for illness treatment [9].

* Corresponding author: Tchinmi Elisabeth; Phone: (+237) 697187867; E-mail address: tchinmie@yahoo.fr

Asparagus africanus Lam. is a plant which belongs to the family of Asparagaceae [10]. The aqueous and ethanolic extracts of the roots of *A. africanus* are antidiabetic, less toxic and rich in phenolic compounds, a potential source of natural antioxidants which could have a great therapeutic importance in the oxidative stress linked to degenerative diseases [11]. *A. africanus* is widely used in traditional medicine as an anti-inflammatory [12] for the treatment of nervous disorders and insomnia [13]. The objective of this work was therefore to assess the sedative and hypnotic effects of *A. africanus* on white mice (*Mus musculus* Swiss).

2. Material

2.1. Plant material

The roots of *A. africanus* were collected in the locality of Bini-Dang, in the Adamawa region (Cameroon). A sample supporting document has been deposited at the headquarters at the national herbarium in Yaoundé (Cameroon) under number 40168/HHC/Cam. The harvested roots of *A. africanus* were washed, dried at room temperature and then ground. 100 ml of distilled water was added to 10 g of this powder and then brought to boil for 20 minutes on a hot plate set at 100 °C. After cooling, the solution was filtered using Wattman number 1 filter paper, then evaporated in an oven (70 °C) for 24 h. 65 ml of distilled water was added to the dry extract obtained constituting the mother solution, the dose of which is 254 mg/kg. Dilution with distilled water was made to 1/2 and 1/4 to obtain the 127 and 63.5 mg/kg doses respectively.

2.2. Animal material

White mice (*Mus musculus* Swiss) of both sexes, weighing 20 to 28 g were used for the various tests. These mice were obtained at the National Veterinary Laboratory (LANAVET) of Garoua (North Cameroon) and further raised in a controlled environment (12 hours of darkness), with access to unlimited food and water. All experiments were performed in accordance with the Guide to the Care and Use of Laboratory Animals published by "National Institutes of Health of the United States" (NIH Publication No. 85-23, revised in 1996). In addition, the study protocol for the handling of animals and the procedure for the experiment were approved by the National Ethics Committee of Cameroon (Ref. No. FW-IRB00001954).

2.3. Drugs

Pentobarbital sodium (Sigma Chemical, USA); Diazepam (DZP), bicuculline (BIC), flumazenil (RO 151788), N-methyl-ß-carboline-3-carboxamide (FG 7142) (Sanofi-Synthelabo). All other chemicals and reagents used in the evaluation of the amount gamma-aminobutyric acid (GABA) and gamma-aminobutyric acid transaminase (GABA-T) in the brain are from Sigma Chemical, USA.

3. Methods

3.1. Sleep potentiation test with diazepam

The method used is that described by Beretz [14] and modified by Rakotonirina [15]. It consisted in inducing sleep in mice after intraperitoneal (i.p.) injection of DZP 50 mg/kg administration dose one hour after administration of 10 ml/kg doses of the roots of *A. africanus* decoction (63.5, 127 and 254 mg/kg) and distilled water for the control batch. Within 2 to 5 min the mice lying on the side, eyelids closed, were asleep. This was characterized by the loss of the righting reflex, observed by tickling the mouse inner pavilion ear using horsehair. The sleep duration was the time that elapses between the moment when the mouse loses the righting reflex and that when the latter reappears (observed when the movement of the foreleg on the side of the tickled ear) [15].

3.2. Sleep induction test with pentobarbital sodium

Three batches of five mice had received 10 ml/kg the roots of *A. africanus* decoction at different doses (63.5, 127 and 254 mg/kg). The mice in the positive control batch had received 10 ml/kg of 3 mg/kg diazepam dose (i.p.) and the mice in the negative control batch had received distilled water. One hour later, a 42 mg/kg sodium pentobarbital dose (i.p.) was administered to each mouse to induce sleep. Sleep onset latency period and sleep duration were recorded. The interval between loss and recovery of the righting reflex was used as an index of hypnotic effect [16]. In antagonistic experiments, N-methyl-ß-carboline-3-carboxamide (FG7142, 10 mg/kg, i.p.), a partial reverse agonist of benzodiazepine of the GABAA receptor complex, flumazenil (RO151788, 10 mg/kg, i.p.), a specific benzodiazepine antagonist in the GABAA receptor complex and bicuculline (BIC, 5 mg/kg, i.p.), a competitive photosensitive antagonist of GABAA receptors, were injected 15 minutes before the start of the administration of the various treatments (extract,

distilled water and diazepam). The treatments were administered 1 hour before the sodium pentobarbital, the latency period and the duration of sleep were recorded [17].

3.3. Gamma aminobutyric acid (GABA) amounts

The amount of GABA in the hippocampus of mice was evaluated by the colorimetric technique of mouse brain homogenates described by Lowe [18]. The working reagent consisted of a mixture of 0.2 ml of 0.14 M ninhydrin solution prepared in a bicarbonate buffer solution (0.5 M; pH 9.9), and 0.1 ml of glacial trichloroacetic acid (TCA) 10%. A 100 µl homogenate sample was taken and introduced into the working reagent, the mixture was incubated at 60 °C in a water bath for 30 minutes. After cooling, the mixture was added into 5 ml of copper tartrate solution prepared from 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid. The whole mixture was kept at a temperature of 25 °C for 10 minutes. The fluorescence resulting from the reaction between ninhydrin and GABA in the basic medium was measured using a spectrofluorimeter and was proportional to the concentration of GABA in the homogenates. A standard GABA solution was prepared parallelly from different GABA masses (50, 100, 150, 200, 250, 300, 350 and 400 µg) which were each mixed with 1.5 mg of glutamate dissolved in 0.1 ml of 10% TCA. The concentration of GABA in the samples was determined by referring to the GABA calibration curve [19]. The content of GABA in the brain was expressed in µg/g of brain tissue.

3.4. Determination of gamma aminobutyric acid transaminase (GABA-T)

The activity of GABA-T was evaluated by the colorimetric assay method of Nayak and Chatterjee [20]. 15 µmol of α-oxoglutarate, 15 µmol of GABA, 10 µg of pyridoxal phosphate, 0.1 ml of homogenate brain supernatant and 0.1 ml of 5% methanol were introduced in the tubes. The final volume of the mixture was made up to 3 ml with Tris-HCl buffer. The tubes were incubated at 37 °C for 30 minutes. The reaction was completed by adding 0.5 ml of 20% glacial TCA. Just before recording, the absorbance of each sample was recorded at 610 nm after 30 and 90s against a blank just after adding 1ml iron chloride (12% $FeCl_3$). The color of the complex formed by succinic semialdehyde acid and 3-methyl-2-benzothia-zolone-2-hydrazone in the presence of 12% $FeCl_3$ was proportional to the concentration of GABA-T in the homogenates. The activity of GABA-T was estimated in pg/min/g of tissue according to the Beer-Lambert law.

3.5. Statistical analysis

The statistical analysis was carried out using GraphPad Prism software version 8.0.1. The results were expressed as mean ± standard error (S.E.M). The different values were compared using analysis of variance (ANOVA) and Tukey's multiple comparison.

4. Results

4.1. Effect of the roots of *Asparagus africanus* decoction on latency period of onset and sleep time by diazepam

Table 1 shows that: *A. africanus* significantly reduced the latency period of onset [($F(3, 16) = 20.22$; $P < 0.001$); $R^2 = 79.13\%$] and increased the sleep time [($F(3, 16) = 2765$; $P < 0.001$); $R^2 = 99.81\%$] induced by diazepam a dose-dependent manner compared to the negative control.

Table 1 Effect of the roots of *Asparagus africanus* decoction on latency period of onset of sleep and sleep time induced by diazepam

Treatment	Dose (mg/kg)	Sedative action	
		Latency period of onset of sleep (min)	Sleep time (min)
DW	-	5.39 ± 0.46	13.15 ± 1.21
Aa	63.5	2.80 ± 0.51***	57.22 ± 0.63***
Aa	127	2.86 ± 0.43***	53.79 ± 0.94***
Aa	254	3.22 ± 0.92***	56.65 ± 0.77***

The results are expressed as mean ± S.E.M, n = 5. *** p < 0.00,1 significant difference compared to the negative control. DW: negative control consisting of mice treated with distilled water, Aa: *Asparagus africanus*.

4.2. Effect of the roots of *Asparagus africanus* decoction on the latency period of onset of sleep and sleep time induced by pentobarbital sodium

Table 2 shows a significant decrease in the latency period of onset [(F (4, 20) = 94.41; P <0.001); R^2 = 94.97%] and a significant increase of sleep time [(F (4, 20) = 2477; P <0.001); R^2 = 99.80%] of sodium pentobarbital-induced dose-dependent for *A. africanus*, comparable to diazepam (positive control) compared to the negative control. This decrease and increase is also significant respectively for the latency period of onset of sleep [(F (4, 36) = 0.277; P <0.05)] and for sleep time [(F (4, 36) = 1.45; P <0.05)] in presence of flumazenil and of bicuculline, comparable to diazepam (positive control) in comparison to the negative control.

Table 2 Effect of the roots of *Asparagus africanus* decoction on the latency period of onset of sleep and the duration of sleep induced by sodium pentobarbital

Treatment	Dose (mg/kg)	Sedative action	
		Latency period of onset of sleep (min)	Total sleep time (min)
DW	-	6.22 ± 0.45	12.95 ± 1.16
DW + FG 7142	- + 10	7.18 ± 0.49	10.91 ± 1.67
DW + RO 151788	- + 10	6.59 ± 0.69	11.72 ± 1.74
DW + BIC	- + 5	7.08 ± 0.64	11.94 ± 1.70
Aa	63.5	3.08 ± 0.20***	55.17 ± 0.92***
Aa	127	2.65 ± 0.33***	56.78 ± 0.44***
Aa	254	2.98 ± 0.39***	56.01 ± 1.02***
Aa + FG 7142	63.5 + 10	6.70 ± 0.16	11.17 ± 0.84
Aa + RO 151788	63.5 + 10	5.84 ± 0.06*	14.53 ± 1.25*
Aa + BIC	63.5 + 5	6.34 ± 0.25*	14.78 ± 1.77*
DZP	3	2.86 ± 0.30***	56.66 ± 0.58***
DZP + FG 7142	3 + 10	6.73 ± 0.28	11.56 ± 0.54
DZP + RO 151788	3 + 10	5.81 ± 0.56*	14.46 ± 1.44*
DZP + BIC	3 + 5	6.33 ± 0.03*	14.77 ± 1.72*

The results are expressed as mean ± S.E.M, n = 5. * p < 0.05, *** p < 0.001, significant difference compared to the negative control. DW: negative control consisting of mice treated with distilled water. DZP: positive control consisting of mice treated with diazepam (3 mg/kg), FG 7142: N-methyl-ß-carboline-3-carboxamide, RO 151788: flumazenil, BIC: bicuculline, Aa: *Asparagus africanus*.

4.3. Effect of a decoction of the roots of *Asparagus africanus* on the concentration of gamma aminobutyric acid in the hippocampus of mice induced by diazepam

Table 3 shows that: *A. africanus* increased significantly in the concentration of gamma aminobutyric acid [(F (3, 16) = 201.7; P <0.001); R^2 = 99.34%] and decreased significantly in concentration of gamma aminobutyric acid transaminase [(F (3, 16) = 41.23; P <0.001); R^2 = 96.87%] induced by diazepam a dose-dependent manner compared to the negative control.

Table 3 Effect of the roots of *Asparagus africanus* decoction on concentration of GABA and activity of GABA-T induced by diazepam

Treatment	Dose (mg/kg)	Concentration of GABA and activity of GABA-T	
		GABA (µg/g)	GABA-T (pg/min/g)
DW	-	241.47 ± 9.14	118.38 ± 12.86
Aa	63.5	377.95 ± 4.94***	42.98 ± 5.24***
Aa	127	364.40 ± 5.89***	61.29 ± 3.07**
Aa	254	337.26 ± 2.73***	91.44 ± 3.45*

The results are expressed as mean ± S.E.M, n = 5. * p < 0.05, ** p < 0.01 *** p < 0.001, significant difference compared to the negative control. DW: negative control consisting of mice treated with distilled water, *Aa: Asparagus africanus.*

4.4. Effect of the roots of *Asparagus africanus* decoction on the concentration of gamma aminobutyric acid in the hippocampus of mice induced by sodium pentobarbital

Table 4 shows a significant increase in the concentration of gamma aminobutyric acid [(F (4, 20) = 38.20; P <0.001); R^2 = 96.83%] and a significant decrease in concentration of gamma aminobutyric acid transaminase [(F (4, 20) = 42.18; P <0.001); R^2 = 97.12%] for *A. africanus* comparable to diazepam (positive control) compared to the negative control. *A. africanus* increase significantly the concentration of gamma aminobutyric acid [(F (4, 36) = 1.16; P <0.05)] and significantly decreases the concentration of aminobutyric acid transaminase [(F (4, 36) = 0.92; P <0.05)] in presence of flumazenil and bicuculline comparable to diazepam (positive control) compared to the negative control.

Table 4 Effect of a decoction of the roots of *Asparagus africanus* on the concentration of gamma aminobutyric acid and gamma aminobutyric acid transaminase induced by sodium pentobarbital.

Treatment	Dose (mg/kg)	GABA (µg/g)	GABA-T (pg/min/g)
DW	-	229.87 ± 24.88	92.93 ± 7.32
DW + FG 7142	- + 10	215.87 ± 12.04	100.98 ± 6.13
DW + RO 151788	- + 10	216.42 ± 4.45	103.50 ± 4.00
DW + BIC	- + 5	225.83 ± 12.08	107.05 ± 3.98
Aa	63.5	362.02 ± 1.79***	30.26 ± 2.92***
Aa	127	345.17 ± 5.58***	41.52 ± 3.00***
Aa	254	320.22 ± 5.15***	36.97 ± 8.07***
Aa + FG 7142	63.5 + 10	220.13 ± 5.13	94.57 ± 7.29
Aa + RO 151788	63.5 + 10	240.22 ± 6.73*	85.59 ± 6.78*
Aa + BIC	63.5 + 5	250.72 ± 3.70*	87.58 ± 3.10*
DZP	3	370.00 ± 12.73***	32.55 ± 5.03***
DZP + FG 7142	3 + 10	223.02 ± 3.19	95.22 ± 4.22
DZP + RO 151788	3 + 10	240.75 ± 3.70*	92.79 ± 7.70
DZP + BIC	3 + 5	251.89 ± 3.61*	92.09 ± 3.71

The results are expressed as mean ± S.E.M, n = 5. * p ≤ 0.05, *** p ≤ 0.001 significant difference compared to the negative control. DW: negative control consisting of mice treated with distilled water. DZP: positive control consisting of mice treated with diazepam (3 mg/kg), FG 7142: N-methyl-ß-carboline-3-carboxamide, RO 151788: flumazenil, BIC: bicuculline, Aa: *Asparagus africanus*.

5. Discussion

A. africanus roots decoction decrease in the latency period of onset of sleep and also significantly increased the duration of sleep induced by diazepam and sodium pentobarbital, which suggests sedative activity [21]. The sedative and hypnotic effects of a substance are recognized by its ability to promote falling asleep and, in general, to prolong the duration of sleep. The quality of sleep obtained is generally close to natural sleep [22]. Benzodiazepines and barbiturates are known to have sedative and hypnotic properties because of their ability to potentiate the duration of sleep [21, 23, 17]. *A. africanus* roots decoction acted in the same way, it would therefore have sedative and hypnotic properties.

Flumazenil, a specific antagonist at the benzodiazepine site in the GABAA complex receptor, blocks by competitive inhibition the effects exerted on the central nervous system by substances which act on the receptors of benzodiazepines. The hypnotic and sedative effects of benzodiazepines are quickly reversed by flumazenil [3]. However, the effect of *A. africanus* on reducing latency and increasing sleep duration was not antagonized by it. Bicuculline, a competitive antagonist sensitive to GABAA receptors, whose role is to block the inhibitory action of GABA receptors, has also not prevented this effect. *A. africanus* would therefore act directly on the GABAergic system [24, 25, 8, 17].

Benzodiazepines and barbiturates act directly on GABAergic receptors and interact with GABAA [3]. Binding GABA to the GABAA receptor increases permeability to chloride ions, which stabilizes the resting potential and induces neuronal inhibition. Thus, GABA will activate inhibitory receptors and thereby reduce the activity of the postsynaptic cell within a reasonable margin [26]. The sedative (for example diazepam) is known to have a pharmacological action by increasing the content of GABA in the brain [27, 25, 17]. It is also found that a decoction of the roots of *A. africanus* significantly increased the concentration of GABA in the brains of mice.

Other pharmacological mechanisms occurring on the GABAergic pathway are evoked such as: the reduction of the recapture of GABA in the synaptic cleft, by inhibiting the degradation enzymes like GABA-T [3]. *A. africanus* also significantly decreased the concentration of GABA-T in the brains of mice, thereby preventing the breakdown of GABA and increasing its action. This again suggests a sedative action.

Altogether, we suggest that the action of *A. africanus* is correlated with an increase in the concentration of GABA and a decrease in GABA-T in the brain. The effectiveness of most herbal medicines is attributed to various active ingredients in combination. For example, saponins show antagonistic activity against amphetamines, a sedative property [23, 28, 29, 17]. It is therefore likely that *A. africanus* has saponins which contributes in part to the effects observed on the central nervous system.

6. Conclusion

Based on the above studies we concluded that: *A. africanus* roots decoction might contain bioactive substances which are hypnotic and sedative. These neuropharmacological properties are possibly mediated via GABAergic neurotransmission. This justifies its use in traditional medicine in the treatment of insomnia.

Compliance with ethical standards

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Disclosure of conflict of interest

Tchinmi Elisabeth, Ngah Esther and Ngo Bum Elisabeth declare that they have no conflict of interest.

Statement of ethical approval

The experiment was carried out at the Laboratory of Medicinal plants, Health and Galenic Fomulation, Faculty of Sciences, University of Ngaoundéré in accordance with approval by the National Ethics Committee of Cameroon (Ref. No. FW-IRB00001954).

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