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A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March

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Abstract

In the present study, we examined the anxiolytic and antidepressant effects of the mixture of alpha- and beta-amyrin (AMY), pentacyclic triterpenes isolated from the stem bark resin of *Protium heptaphyllum*. These effects of AMY were demonstrated by the open-field, elevated-plus-maze, rota rod, forced swimming, and pentobarbital-induced sleeping time tests, in mice. In the open-field test, AMY at the doses of 10, 25 and 50 mg/kg, after intraperitoneal or oral administrations, significantly decreased the number of crossings, grooming, and rearing. All these effects were reversed by the pre-treatment with flumazenil (2.5 mg/kg, i.p.), similarly to those observed with diazepam used as a positive standard. In the elevated-plus-maze test, AMY increased the time of permanence and the number of entrances in the open arms. On the contrary, the time of permanence and the number of entrances in the closed arms were decreased. All these effects were also completely reversed by flumazenil, an antagonist of benzodiazepine receptors. In the pentobarbital-induced sleeping time test, AMY at the same doses significantly increased the animals sleeping time duration. In the rota rod test, AMY did not alter motor coordination and, thus, was devoid of effects, as related to controls. Since AMY, at the doses of 10 and 25 mg/kg, showed a sedative effect in the open field test, lower doses (2.5 and 5.0 mg/kg) were used in the forced swimming test, producing a decrease in the immobility time, similarly to that of imipramine, the positive control. The effect of AMI was greater when it was administered 15 min after imipramine (10 mg/kg). However, the antidepressant AMY effects were not altered by the previous administration of paroxetine, a selective blocker of serotonin uptake. In addition, AMY effects in the forced swimming test were totally blocked by reserpine pretreatment, a drug known to induce depletion of biogenic amines. In conclusion, the present work evidenced sedative and anxiolytic effects of AMY that might involve an action on benzodiazepine-type receptors, and also an antidepressant effect where noradrenergic mechanisms will probably play a role.

Keywords: *Protium heptaphyllum*; Alpha-and beta-amyrin; Sedative; Anxiolytic; Antidepressant

1. Introduction

Protium heptaphyllum is a medicinal plant largely found in the North and Northeast Brazil. It is popularly known as "almécega" and "breu branco", and used for inflammations, pain, ulcers and wounds (Correia, 1984). The resin extracted from its bark is rich in triterpenes, such as alpha- and beta-amyrin (AMY). Triterpenes isolated from several species of medicinal plants are, in general, responsible at least in part for their biological activities. Thus, the mixture of alpha- and beta-amyrin (1:2) was isolated from *P. heptaphyllum* (Susunaga et al., 2001), and described as having antiinflammatory and analgesic properties (Miranda et al., 2000; Aragão et al., 2002). The antiinflammatory activity of AMY was also reported to be dependent upon its inhibitory activity on protein kinases from eucaryotic cells (Hasmeda et al., 1999). Recent studies demonstrated that the mixture of alpha- and beta-amyrin presents antiinflammatory as well as gastroprotector activities, in mice and rats (Oliveira et al., 2004). Another study showed a protector effect of alpha- and beta-amyrin against hepatic

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lesions provoked by paracetamol, in mice (Oliveira et al., 2005b). Furthermore, the antinociceptive properties of alpha- and beta-amyrin were also demonstrated to be seemingly the result of the participation of protein kinase C and protein kinase A (Otuki et al., 2001, 2005). Studies carried out with beta-amyrin palmitate, isolated from *Lobelia inflata,* showed that this triterpene possesses a central sedative as well as an anti-depressant activity, in mice, at the doses of 5, 10 and 20 mg/kg (Subarnas et al., 1993). These authors demonstrated that the antidepressant activity of beta-amyrin palmitate, at the doses of 2.5, 5 and 10 mg/kg, i.p., presented a noradrenergic mechanism of action, possibly inhibiting alpha1 adrenoceptors (Subarnas et al., 1993). The objective of the present work was to analyze the effects produced by the acute administration of the mixture of alpha- and beta-amyrin from *P. heptaphyllum*, as assessed by the open field, elevated-plus-maze, pentobarbital-induced sleeping time, rota rod, and forced swimming tests, in order to evaluate the sedative, anxiolytic, and antidepressant activities of these triterpenes, attempting to clarify their mechanism of action.

2. Materials and methods

2.1. Plant material

The plant was collected in September 1998, at the city of Crato, state of Ceará, Brazil, and was identified by Prof. A. G. Fernandes, from the Department of Biology of the Federal University of Ceará. The voucher specimen is deposited at the Prisco Bezerra Herbarium under the number 28509.

2.2. Animals

Male Swiss mice (20–30 g) from the Animal House of the Federal University of Ceará were used throughout the experiments. Animals were housed in standard environmental conditions (23±2 °C, humidity 60±5%, 12 h light–12 h dark cycle), with free access to a commercial diet and water. Each animal was used only once. Control animals were administered with the suspension of 0.5% Tween 80, in distilled water used as vehicle. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services, Washington DC, 1985. The project was previously approved by the Animal's Ethics Committee, of the Faculty of Medicine of the Federal University of Ceará.

2.3. Isolation of alpha- and beta-amyrin (AMY)

To obtain the resin, incisions were made on the plant stem. The resin (20 g) was fractionated by silica gel column chromatography with hexane, chloroform, ethyl acetate and methanol. Fractions obtained with chloroform (5.2 g) were repeatedly chromatographed on silica gel, and eluted with increasing amounts of hexane–ethyl acetate. Fractions obtained from hexane–ethyl acetate (1:1) were analyzed by TLC, and gave as result 450 mg of alpha- and beta-amyrin (Fig. 1). The

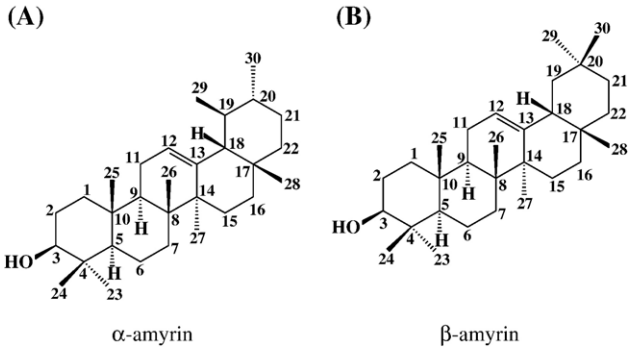

Fig. 1. Chemical structures of (A) alpha-amyrin (3β-hydroxyurs-12-eno) and (B) beta-amyrin (3β-hydroxyolean-12-eno) isolated from the crude resin of *Protium heptaphyllum.*

fraction rich in alpha- (67%) and beta-amyrin (33%) was used for further purification. The identification of these isomers was made by infrared spectrophotometry (KBr) ν_{max} cm^{-1} (3300, 1480 and 1050); NMR ^{1}H (500 MHz, $CDCl_3$); ^{13}C (125 MHz, $CDCl_3$) and melting point 179–181 °C, according to the literature (Mahato and Kundu, 1994). The mixture of alpha- and beta-amyrin is a white amorphous powder, presenting a slight odor, low aqueous solubility, but soluble in organic solvents. In the present work, the mixture was suspended in 0.5% Tween 80 distilled in water, and sonicated before use.

2.4. Reagents and drugs

Pentobarbital and reserpine sulphate were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Tween 80 was from Vetec Química Farm. Ltda (Rio de Janeiro, Brazil). Diazepam and Flumazenil from Cristália Prod. Química Farm. Ltda (São Paulo, Brazil). Imipramine from Novartis Biociências S.A. (São Paulo, Brazil), Paroxetine from Glaxo Smith Kline Brasil Ltda. (Rio de Janeiro, Brazil). All other drugs were of analytical grade.

2.5. Pharmacological tests

2.5.1. Open-field test

The open-field arena was made of acrylic (transparent walls and black floor, 30×30×15 cm), divided into nine squares of equal areas. The open-field was used to evaluate the exploratory activity of the animal (Archer, 1973). The mouse was placed individually into the center of the arena, and allowed to explore it freely. The observed parameters were: ambulations (the number of squares crossed with all four paws), numbers for grooming and rearing, recorded for the last 5 min of the 6 min testing period.

2.5.2. Barbiturate-induced sleeping time

In this test, performed according to the method of Ferrini et al. (1974), the mouse sleep was induced by an i.p. administration of 40 mg/kg body wt of pentobarbital, and the duration of sleep (min) of each animal was observed. The sleeping time was recorded as the period for recovering the righting reflex.

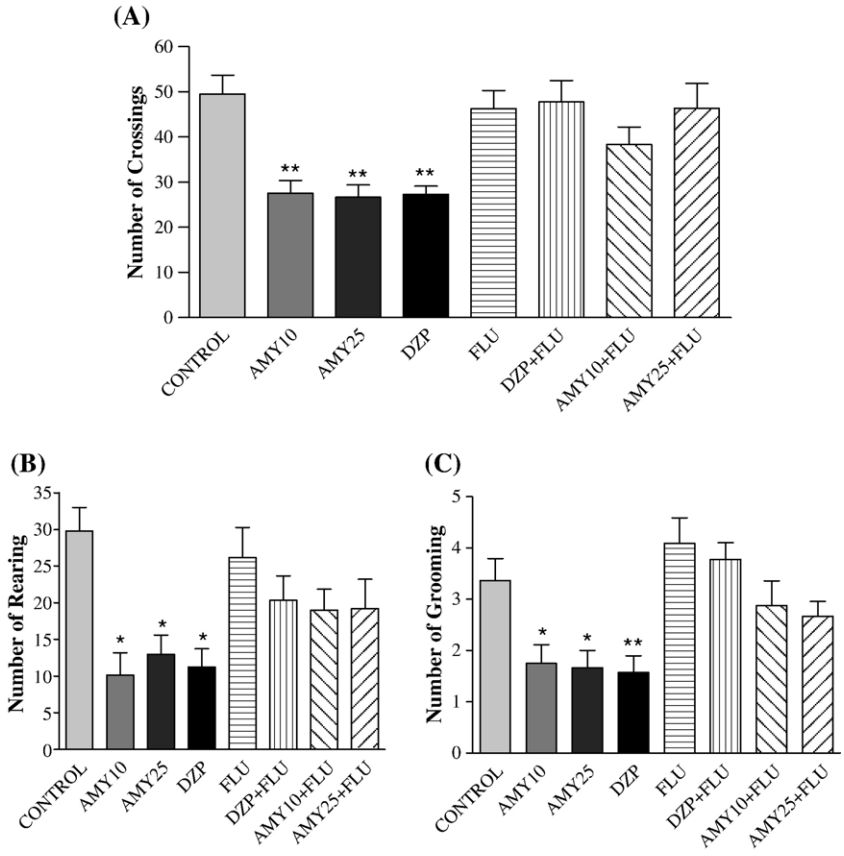

Fig. 2. Effects of alpha- and beta-amyrin (AMY) from *P. heptaphyllum* in the open field test, in mice. A = number of crossings; B = number of rearing and C = number of grooming. Control = Tween 0.5%; AMY, 10 and 25 mg/kg, i.p.; DZP = diazepam, 1 mg/kg, i.p.; FLU = flumazenil, 2.5 mg/kg, i.p. Each bar represents the mean ± SEM, from 6 to 13 animals per group. *$p<0.05$ and **$p<0.01$ as compared to controls (ANOVA and Tukey as the *post hoc* test).

2.5.3. Rota rod

For the rota rod test, the animal was placed with the four paws on a 2.5 cm diameter bar, 25 cm above the floor, which was turning at 12 rpm. For each animal, the number of falls (up to three falls) and the time of permanence on the bar for 1 min were registered (Dunham and Miya, 1957).

2.5.4. Elevated-plus-maze

This test has been widely validated for measuring anxiolytic and anxiogenic-like activities, in rodents (Lister, 1987). The apparatus consisted of two opposite open arms (30 × 5 cm), crossed by two closed arms of the same dimensions, with 25 cm high walls. The arms were connected to a 5 × 5 cm central square. The apparatus was elevated 45 cm above the floor, in a dimly illuminated room. Mice were placed individually in the center of the maze, facing an enclosed arm, and the number of entries and time spent on the open arms were recorded for the next 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

2.5.5. Forced swimming test

This test is the most widely used and recognized pharmacological model, for assessing antidepressant activities. In the present work, we employed that described by Porsolt et al. (1977a,b, 1978)). The development of immobility when mice were placed inside an inescapable cylinder filled with water

Table 1
Effects of alpha- and beta-amyrin (AMY) from *Protium heptaphyllum*, after oral administration to mice, in the open field test

Group	NC/20 min	Number of grooming	Number of rearing
Control (8)	51.88±1.95	4.33±0.60	34.11±2.34
DZP (8)	41.63±2.75*	1.50±0.42**	12.25±2.89**
AMY 10 (8)	46.75±1.83	1.38±0.42*	24.75±2.77
AMY 25 (8)	43.75±2.53**	1.13±0.23**	26.50±2.80
AMY 50 (8)	37.25±1.94*	1.13±0.35**	20.25±3.05**

Experiments were performed as described in Materials and methods. Values are mean±SEM of the number of animals shown in parenthesis. NC = number of crossings. DZP = diazepam used as a positive control. *$p<0.05$ and **$p<0.001$, as compared to controls (ANOVA and Tukey as the *post-hoc* test).

Table 2
Effects of alpha- and beta-amyrin (AMY) from *Protium heptaphyllum*, on the rota rod test, in mice

Group	TP	NF
Control, i.p. (8)	48.38±6.43	2.5±0.19
AMY 10 mg/kg, i.p. (8)	49.63±4.71	2.12±0.29
AMY 25 mg/kg, i.p. (8)	55.25±3.63	1.12±0.35

Experiments performed as described in Materials and methods. Values are means±SEM of the number of animals specified in parentheses. TP = time of permanence; NF = number of falls.

Table 3
Effects of alpha- and beta-amyrin (AMY) from *Protium heptaphyllum*, on the barbiturate-induced sleeping time test, in mice

Group	Sleeping time (min)	Increase (%)
Control, p.o. (13)	48.5±4.1	–
AMY 10 mg/kg, p.o. (12)	87.5±3.5*	80.4
AMY 25 mg/kg, p.o. (12)	77.8±6.4*	60.4
Control, i.p. (10)	54.0±4.5	–
AMY 10 mg/kg, i.p. (12)	78.5±3.5*	45.4
AMY 25 mg/kg, i.p. (10)	73.5±4.8*	36.1

Experiments performed as described in Materials and methods. Values are means±S.E.M. of the sleeping time, measured after the pentobarbital injection. In parenthesis is the number of animals per group. *$p<0.05$ as compared to controls (ANOVA and Tukey as the *post hoc* test).

reflects the cessation of persistent escape-directed behavior. Briefly, mice had a swimming-stress session for 15 min (pre-test), 24 h before being individually placed into glass cylinders (height: 25 cm; diameter: 10 cm; containing 10 cm of water at 24±1 °C) for 5 min (test). A mouse was judged to be immobile when it ceased struggling and remained floating motionless on the water, making only small movements necessary to keep its head above water.

2.5.6. Experimental protocol

Animals were treated with AMY and submitted to the elevated-plus-maze test, 30 (i.p.) or 60 (p.o.) min later, followed by the open-field and rota rod tests. Another group of animals was submitted to the forced swimming test. Controls were administered with distilled water.

In order to elucidate the mechanisms possibly involved with the sedative and anxyolitic effects of alpha- and beta-amyrin, we used diazepam and flumazenil administered alone or associated with AMY, in the open-field and elevated-plus-maze tests. Similarly, in order to clarify the antidepressant effect of AMY, imipramine and paroxetine alone or in combination with AMY were used, in the forced swimming test. Imipramine was chosen because this drug is a classical antidepressant that acts through noradrenergic and serotonergic pathways. Paroxetine, another antidepressant drug, is more selective, and acts predominantly through serotonergic pathways. Both antidepressants were then used, alone as positive controls or in combination with two

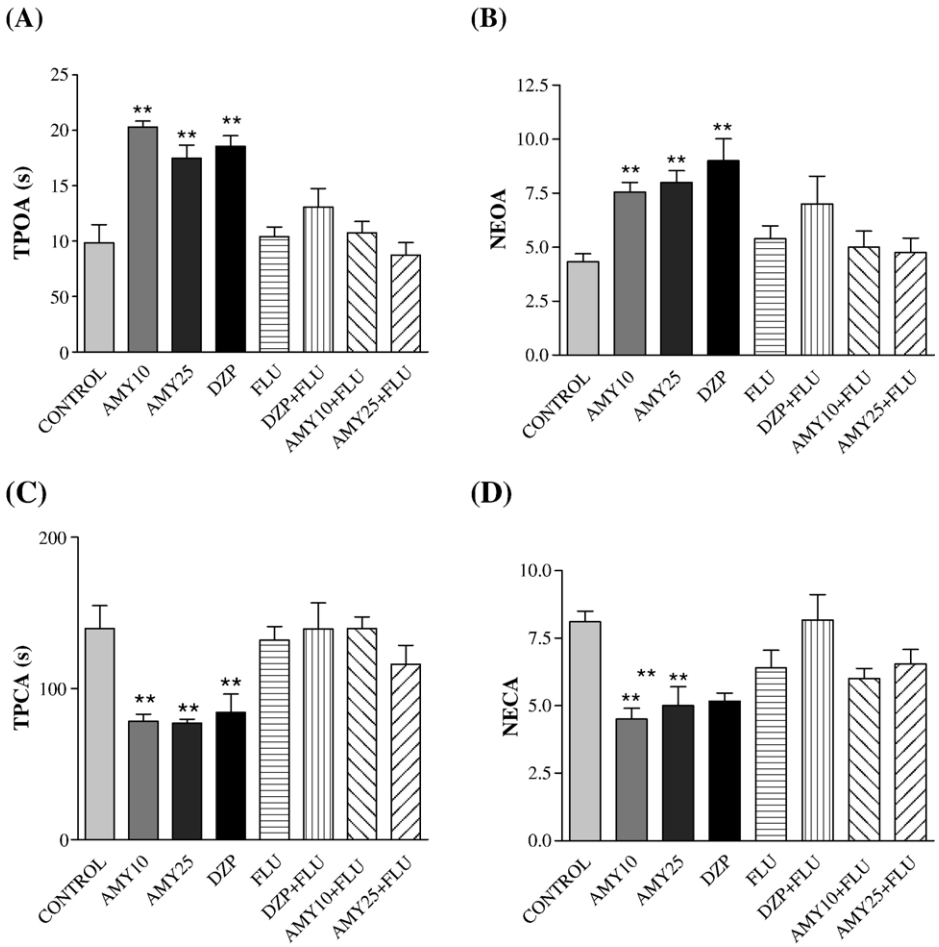

Fig. 3. Effects of alpha- and beta-amyrin (AMY) from *P. heptaphyllum* in the elevated plus maze test, in mice. A=time of permanence in the open arms (TPOA); B=number of entrances in the open arms (NEOA); C=time of permanence in the closed arms (TPCA); and D=number of entrances in the closed arms (NECA). Control=Tween 0.5%; AMY, 10 and 25 mg/kg, i.p.; DZP=diazepam, 0.5 mg/kg, i.p.; FLU=flumazenil, 2.5 mg/kg, i.p. Each bar represents the mean ± SEM, from 6 to 12 animals per group. **$p<0.01$ as compared to controls (ANOVA and Tukey as the *post hoc* test).

lower doses of AMY, in order to investigate any possible alteration/interference of imipramine or paroxetine on the antidepressant effect of AMY. Reserpine, a drug known to cause depletion of biogenic amines (noradrenaline, dopamine and serotonin) from storage granules, was also used to evaluate the participation of those amines in the AMY antidepressant effect. In the combination protocol, imipramine, paroxetine or reserpine were administered 10 min before AMY, and the test was performed 30 min later.

2.6. Statistical analysis

All data represent mean±S.E.M. values. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey as the *post hoc* test. All analyses were performed using the software Prism 3.0 for Windows. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

3. Results

AMY, at the doses of 10 and 25 mg/kg, i.p., showed sedative effects as assessed by the open-field test, in mice (Fig. 2). Significant effects were detected with both doses which produced similar percentages of inhibition (44 and 46%, respectively) in the number of crossings ($q=4.166$, $p<0.01$; $q=3.908$, $p<0.01$), as related to controls. The number of rearing ($q=3.891$, $p<0.01$; $q=2.992$, $p<0.05$) and the time spent in grooming behavior ($q=2.806$, $p<0.05$; $q=2.702$, $p<0.05$) were reduced with AMY 10 and AMY 25 mg/kg respectively, whose results were similar to those observed with diazepam, used as a positive control (no. of crossings, $q=3.697$, $p<0.01$; rearing, $q=3.815$, $p<0.01$; grooming, $q=3.594$, $p<0.01$). Flumazenil was used for evaluating the possible mechanism of action of the sedative effect of AMY. For this, 15 min after the flumazenil injection, AMY (10 and 25 mg/kg) was administered, and under these conditions the animals' behavior was similar to that of controls, indicating that AMY presents a benzodiazepine-type of sedative effect.

Similar effects, although lower (16 and 28% inhibition of the number of crossings), were observed after AMY orally administered, at the doses of 25 ($q=3.640$, $p<0.05$) and 50 ($q=6.552$, $p<0.001$) mg/kg, in the open field test (Table 1). Significant effects (around 70% inhibition) on the time spent in grooming behavior were observed with the doses of 10 ($q=6.927$, $p<0.001$), 25 ($q=7.512$, $p<0.001$) and 50 mg/kg ($q=7.515$, $p<0.001$), p.o., as well as in the number of rearings where a 40% inhibition was seen with the dose of 50 mg/kg ($q=5.104$, $p<0.001$).

In the rota rod test, used for evaluating motor coordination and presence of any muscle relaxation effect, there was no change after AMY administration (10 and 25 mg/kg, i.p.), as compared to controls (Table 2).

In the barbiturate-induced sleeping time test, the oral ($q=7.157$, $p<0.001$; $q=4.332$, $p<0.01$) as well as the intraperitoneal ($q=5.286$, $p<0.01$; $q=5.579$, $p<0.01$) administration of AMY (10 and 25 mg/kg respectively) increased the sleep latency time in 80 and 60%, and in 45 and 36%, respectively, suggesting a potentiation of the pentobarbital effect (Table 3).

A possible anxyolitic activity of AMY (10 and 25 mg/kg, i.p.) was assessed by the elevated-plus-maze test (Lister, 1987). Results (Fig. 3) showed that the time of permanence in the open arms, TPOA ($q=6.622$, $p<0.01$; $q=4.186$, $p<0.01$), and the number of entrances in the open arms, NEOA ($q=3.483$, $p<0.01$; $q=3.350$, $p<0.01$), were significantly increased, in 105 and 77%, and in 75 and 85%, respectively, as compared to controls. On the contrary,

Table 5
Antidepressant effects of alpha- and beta-amyrin (AMY, i.p.) from *Protium heptaphyllum* in the forced swimming test, in mice, and the possible involvement of noradrenergic and/or serotonergic systems

Group	Time of immobility (s)	% Change
Control (21)	203.5±7.8	–
AMY 1 mg/kg (6)	162.2±17.7	−20
AMY 2.5 mg/kg (9)	145.9±13.2*	−28
AMY 5 mg/kg (10)	91.3±7.8**	−55
IMI 10 mg/kg (6)	145.7±12.6*	−28
IMI 30 mg/kg (8)	73.9±13.3**	−64
PAROX 4 mg/kg (20)	75.6±17.9	Ns
PAROX 16 mg/kg (18)	145.9±6.9**	−28
AMY 1+IMI 10 (09)	114.9±14.6**	−44
AMY 2.5+IMI 10 (07)	102.4±8.5**	−50
AMY 2.5+PAROX 4(14)	150.4±14.0*	−26
RESERP 2 mg/kg (7)	246.6±9.8	+2
RESERP2+AMY2.5(06)	197.0±13.3	Ns

Experiments performed as described in Materials and methods. AMI was administered intraperitoneally. Values are mean±S.E.M. of the immobility time. In parenthesis is the number of animals per group. IMI=imipramine; PAROX=paroxetine; RESERP=reserpine. Drugs were administered 10 min before AMY and the test performed 30 min later. *$p<0.05$ and **$p<0.01$ as compared to controls (ANOVA and Tukey as the *post hoc* test).

Table 4
Effect of alpha- and beta-amyrin (AMY) orally administered to mice, in the elevated-plus-maze test

Group	NEOA	NECA	TPOA	TPCA
Control (10)	154.6±10.92	8.75±0.88	154.6±10.92	102.2±7.91
DZP (10)	217.2±14.44**	4.13±1.19**	219.9±16.81**	59.7±11.07**
AMY (10) 10 mg/kg	198.4±9.39 *	6.11±0.754*	198.4±9.39**	73.3±9.06*
AMY (9) 25 mg/kg	208.3±10.70**	4.67±0.62**	216.5±7.84**	55.9±6.17**
AMY (10) 50 mg/kg	209.1±11.80**	4.33±0.69**	17.0±9.80**	52.6±5.27**

Experiments performed as described in Materials and methods. Values are mean±S.E.M of the number of animals in parenthesis. DZP=diazepam; NEOA=number of entrances into the open arms; NECA=number of entrances into the closed arms; TPOA=time of permanence in the open arms; TPCA=time of permanence in the closed arms. *$p<0.05$ and **$p<0.01$ as compared to controls (ANOVA and Tukey as the *post hoc* test).

Table 6
Effect of alpha- and beta-amyrin (AMY) orally administered to mice, in the forced swimming test

Group	Immobility time (s)
Control (8)	190.0±5.51
AMY 1 mg/kg (8)	166.4±12.12
AMY 2.5 mg/kg (8)	117.1±20.46 *
AMY 5 mg/kg (8)	105.3±16.10 *
IMI 30 mg/kg (8)	85.1±13.72 **

Experiments performed as described in Materials and methods. Values are mean ±SEM of the immobility time. In parentheses is the number of animals per group. IMI=imipramine as a positive standard. *$p<0.01$ as compared to controls; **$p<0.001$ as compared to controls (ANOVA and Tukey as the test *post-hoc*).

AMY (10 and 25 mg/kg, i.p.) significantly reduced the time of permanence in the closed arms, TPCA ($q=3.832$, $p<0.01$; $q=3.291$, $p<0.01$) by 44 and 45%, as well as the number of entrances in the closed arms, NECA ($q=4.888$, $p<0.01$; $q=3.502$, $p<0.01$) by 44 and 38%, respectively. These effects were similar to those of diazepam (TPOA, $q=5.210$, $p<0.01$; NEOA, $q=4.719$, $p<0.01$; TPCA, $q=3.360$, $p<0.01$; NECA, $q=3.498$, $p<0.01$). Flumazenil (2.5 mg/kg, i.p.) reversed the effects of diazepam and of AMY at both doses, although showing no effect alone. Similar effects were observed in the elevated-plus-maze test after the oral administration of AMY, whose data were very close to those observed with diazepam. Furthermore, increases in TPOA and NEOA as well as decreases in TPCA and NECA were demonstrated with all doses (Table 4).

The possible antidepressant effect of AMY after intraperitoneal or oral administration was studied in the forced swimming test (Tables 5 and 6). Under this condition, AMY was used at smaller doses (1, 2.5 and 5 mg/kg, i.p.), since at the doses of 10 and 25 mg/kg the antidepressant effect is masked by the sedative and anxiolytic effects of AMY (data not shown). The results showed that AMY presents a significant antidepressant effect, at the doses of 2.5 and 5 mg/kg, suggested by the decrease in 28 and 55%, respectively, of the time of immobility. The smallest dose (1 mg/kg) was devoid of any significant effect. The association of AMY, at the doses of 1 and 2.5 mg/kg, with imipramine (IMI) showed a greater decrease of the immobility time ($q=4.729$, $p<0.01$ and $q=4.924$, $p<0.01$), respectively, as related to the groups treated with AMY alone (1 mg/kg, $q=1.899$, $p>0.05$ and 2.5 mg/kg, $q=3.075$, $p<0.05$) or IMI ($q=2.657$, $p>0.05$) alone. However, the association of AMY with paroxetine ($q=3.271$, $p<0.05$) did not alter the effect observed with AMY or paroxetine alone ($q=3.075$, $p<0.05$ and $q=1.903$, $p>0.05$), respectively, suggesting that the serotonergic system is not involved in the antidepressant effect of AMY. On the contrary, the AMY activity was totally blocked by the previous administration of reserpine. These data suggest that the noradrenergic system participates in the AMY antidepressant action. Significant decreases in the immobility time were also observed after AMY administration (p.o.), at the doses of 2.5 (117.1±20.46 s; $q=5.054$, $p<0.01$) and 5 (105.3±16.10 s; $q=5.876$, $p<0.01$) mg/kg, as compared to controls (190.0±5.505 s).

4. Discussion

In the present work, the central effects of the triterpene isomeric mixture, alpha- and beta-amyrin (AMY) isolated from *P. heptaphyllum*, were studied. AMY was firstly evaluated on the open-field test which gives a good indication of the animal's emotional state. The results showed that AMY was able to significantly decrease not only the number of crossings, indicative of a possible sedative effect, but also grooming and rearing.

Flumazenil reversed not only the diazepam effect but also the AMY effect, indicating that both drugs might present a similar mechanism of action. In order to study the possible anxyolitic effect of AMY, the elevated-plus-maze test was used, and the results showed that AMY was also able to significantly increase the time of permanence as well as the number of entrances in the open arms, indicating a positive response. Our results point out that the sedative as well as the anxyolitic effects of AMY possibly involve the GABA-A receptor complex. A sedative action was already shown by other triterpenes, such as those present in *Glaphinia glauca* (Herrera-Ruiz et al., 2005) and *Centella asiatica* (Brinkhaus et al., 2000; Wijeweera et al., 2006), as assessed by the elevated-plus-maze test in rodents. Active constituents of *C. asiatica* are primarily triterpenoid compounds and, thus, chemically similar to AMY. These triterpenes exhibit anti-anxiety activity that is thought to be due to cholinergic mechanisms. Furthermore, an extract from *C. asiatica* was shown to exert a dose-dependent increase in GABA levels, in rat brain (Chatterjee et al., 1992).

The sedative and anxiolytic effects of AMY were further confirmed by the potentiation of the barbiturate-induced sleeping time. The hypnotic action of pentobarbital was demonstrated by Petty (1995) to be mediated by the GABA-A receptor complex. Accordingly, Subarnas et al., 1993, showed that beta-amyrin palmitate, at the doses of 5, 10 and 20 mg/kg, potentiated pentobarbitone-induced narcosis, in mice. However, their data and ours indicate that the potentiation was not a dose-dependent phenomenon, since the effect observed with the higher dose was less intense.

Numerous neural pathways are involved in the pathophysiology of depression and anxiety states. Therefore, a great number of neurotransmitters participate in the underlying mechanisms of anxiolytic and antidepressant drugs (Palucha and Pile, 2002). It is widely accepted that anxiolytic drugs of the benzodiazepine (BZ) type act clinically by enhancing the effect of GABA, at the GABA-A receptor. Although classical BZ agonists enhance the function of the GABA-A receptor and are effective anxiolytics, they present unwanted side-effects, including sedation, dependence and abuse liability (Whiting, 2006). Recently (Dias et al., 2005; Morris et al., 2006), new data confirmed the critical importance of alpha 2 and 3 GABA-A subtype receptors in mediating BZ anxiolysis. These findings are promising, in terms of anxiolytic efficacy and decreased unwanted effects. This could represent a starting point for the development of a new generation of drugs selective to subtypes of BZ receptors (Whiting, 2006).

Sedative and anxiolytic drugs, such as benzodiazepines, facilitate the action of the gama-aminobutyric acid (GABA)

upon the GABA-A receptor. Clinically, these substances are widely used as sedative and anxiolytic drugs, agreeing with studies in animal models such as the elevated-plus-maze test, where the effects of DZP are highly reproducible. Based on these findings, it could be thought that substances able to reduce animal's anxiety exposed to these paradigms could exert their effects through an action similar to that of benzodiazepines (Herrera-Ruiz et al., 2005). According to other studies (Oliveira et al., 2005a), AMY did not alter mice motor coordination, in the rota rod test.

The forced swimming test is a behavior test which, in rodents, gives an indication of the clinical efficacy of various types of antidepressant drugs. Nowadays, antidepressants are known to act by several distinct mechanisms at the receptor level, probably also stimulating similar pathways at the sub-cellular level (Yildiz et al., 2002). AMY was also able to decrease the immobility time of mice, at the doses of 2.5 and 5 mg/kg, in the forced swimming test. At higher doses (10 and 25 mg/kg), however, the antidepressant effect was masked by sedative and hypnotic actions of the drug (data not shown). The AMY effect was increased by imipramine, a tricyclic antidepressant (TCA) which blocks the reuptake of both serotonin and norepinephrine. However, no alteration was seen after AMY association with paroxetine, a known selective serotonin reuptake inhibitor. Additionally, AMY effects were totally blocked by the reserpine pretreatment, a known inhibitor of the vesicular catecholamine transporter (that facilitates vesicular storage). A similar process occurs at storage sites for 5-HT, what can finally result in a depletion of biogenic amines. Furthermore, this finding suggests that the antidepressant effect of AMY is probably related, at least in part, to the increase in CNS noradrenergic activity.

An earlier work (Subarnas et al., 1993) demonstrated that the beta-amyrin palmitate might stimulate the release of norepinephrine from newly synthesized pools. These authors showed that beta-amyrin palmitate caused the release of norepinephrine from mouse brain synaptosomes. This finding is a proof of the assumption that beta-amyrin palmitate might cause a release of norepinephrine. Thus, the reduction in the immobility time of mice treated with beta-amyrin palmitate might be due to the increase of noradrenergic activity, which is presumably related to the possible antidepressant activity of this compound (Subarnas et al., 1993). Furthermore, in a recent work (Chen et al., 2003), triterpenes from *C. asiatica* were shown to reduce corticosterone levels in serum, and to increase contents of 5-HT, NE and DA as well as their metabolites, in rat brain. The recent preoccupation with the role of serotonin in the treatment of depression has ignored the role of noradrenaline and the fact that these two neurotransmitters do not work in isolation from each other.

Early evidence (Brunello et al., 2002) of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism. Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression. A number of relatively selective noradrenaline reuptake inhibitors have been widely used as antidepressants, including desipramine and protriptyline. However, all these drugs are TCAs, and have a propensity to cause unwanted side effects, due to their non-selective interactions at muscarinic, adrenergic and histaminergic receptors. The efficacy of these drugs is undoubtedly a product of noradrenergic, serotonergic and non-selective receptor affinities.

Our results give support to the idea that AMY interacts with the GABA-A receptor, probably at the receptor subtypes that mediate BDZ effects, to produce sedative and hypnotic activities, and also acts to increase the noradrenergic activity that is the main factor responsible for its antidepressant activity. Additional studies, however, are needed to fully clarify the mechanism of anxiolytic and antidepressant effects of AMY. Furthermore, AMY could manifest these effects at doses not showing either sedative or hypnotic activities, being thus potentially useful in clinical practice.

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