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Research Paper

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SEDATIVE AND ANTICONVULSANT PROPERTIES OF *PASSIFLORA EDULIS* DRIED LEAVES DECOCTION IN MICE

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Abstract

Passiflora edulis Sims is native from Tropical area of South America. It is used in traditional medicine in the treatment of some diseases related to the nervous system and others. The extract of *Passiflora edulis* possesses sedative activity in mice. It significantly increased the total sleep time induced by diazepam (50 mg/kg i.p.). The total sleep time increased from 31 $\pm$ 11 min in the control group to 77.6 $\pm$ 15 and 78.3 $\pm$ 16 min in the groups treated with extract at the doses of 132.5 and 1325 mg/kg respectively. This extract showed also anticonvulsant activity in mice. It protected mice against strychnine -induced seizures and antagonized N-methyl-D-aspartate- induced turning behavior in mice. The ED_{50} for the protection against seizures -induced by strychnine was 320 mg/kg intraperitoneally (i.p.). For N-methyl-D-aspartate -induced turning behavior, the ED_{50} was 300 mg/kg i.p. *Passiflora edulis* extract had less effect against pentylene tetrazol- induced seizures. The potentiation of diazepam-induced sleep, the antagonism of chemical -induced seizures and turning behavior suggest that *Passiflora edulis* extract possess sedative and anticonvulsant properties in animals.

***Keywords*:** Epilepsy; Anticonvulsant; Decoction; Seizures; *Passiflora edulis*

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Introduction

Passiflora edulis Sims is native from Tropical area of South America. It is found in Argentina, Brazil, Paraguay, Peru, etc (Morton, 1987). It belongs to the family of Passifloraceae and is cultivated for its very sweet fruits (Wagner, et a., 1999).

Literature revealed that extensive work has been performed on *Passiflora edulis* Sims. And it has been found to possess therapeutics properties. Flowers of *Passiflora edulis* contain alkaloids and flavonoids and are used in phytotherapy to treat pain, insomnia, anxiety, nervous tension (Wolfman., et al.1994).

Its leaves are used in the treatment of high blood pressure. *Passiflora edulis* and its related species are also used in traditional medicine to treat headache, convulsions, muscular spasms, and as aphrodisiac and antitussive agents (Dhawan.et al., 2002; Dhawan. et al., 2003).

Passiflora edulis is widely used in traditional medicine in the treatment of many diseases. *Passiflora edulis* was collected at Bertoua (Cameroon) and tested to determine its sedative and anticonvulsant properties in mice because in traditional medicine the plant has therapeutic properties for the nervous system diseases. Secondly, in our laboratory, the search to identify and collect all medicinal plants used in the treatment of epilepsy, insomnia, anxiety and head ache is a priority.

Methodology

Animals

Adult male mice (*Mus musculus* Swiss; 20 – 25 g; 6 per group) were used throughout these studies. The animals were housed in standard cages at 25°C on a 12/12 h light-dark cycle. They were supplied with food and water *ad libitum*

Plant material

The plant specimens of *Passiflora edulis* used in these studies were collected in Cameroon in the vicinity of Bertoua. The voucher specimen was deposited at the National Herbarium of Cameroon.

Preparation of extract

The decoction of *Passiflora edulis* was obtained according to a method described below. The dried leafs of *Passiflora edulis* were ground. The powder (10 g) was macerated in 75 ml of distilled water for 1 h. The mixture was boiled for 20 min. After cooling, the supernatant or decoction was collected and filtered. The stock solution obtained (30 ml) correspond to a concentration of 0.1325 g/ml, which represents a 5% yield.

The decoction was administered intraperitoneally (i.p.) 1 h before the test decoction. The following doses were used: 44.2, 132.5, 442 and 1325 mg/kg.

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Pharmacological tests

Diazepam-induced sleep in mice

The method described by Beretz et *al*. 1978 and modified by Rakotonirina et *al*. 2001 was used. Sleep potentiating effects of the plant was studied in mice that received diazepam at a dose of (50 mg/kg) 1 hour after decoction and distilled water administration. Observing time between the disappearing and the recovery of the straightening reflex measured the sleeping time.

N-methyl-D-aspartate (NMDA) test

Mice were injected subcutaneously (s.c.) with NMDA, 75 mg/kg, 1 h after administration of the extract. They were observed for 30 min. Animals that did not exhibit turning behavior within the 30 min observation period were declared protected. Turning was characterized by two consecutive 360° cycles fulfilled by the same animal (Ngo Bum et al. 2002; Schmutz et al., 1990). For the non-protected animals, the onset time of this behavior was recorded. There was two control groups: one with placebo and a positive control group receiving 0.33 ηmol/kg D-AP7, a competitive NMDA antagonist (Croucher et al. 1982).

Strychnine (STR) test

The method has been described previously (Ngo Bum et al. 2001; Ngo Bum et al. 2002). In brief, STR convulsions followed by death were induced in male mice by the i.p. injection of 2.5 mg/kg STR nitrate. A protective effect of the decoction given i.p. 1 h prior to STR was recorded and compared to the one of 3 mg/kg clonazepam. The number of animals, which survived more than 10 min, served as criterion of protection. The time of onset of convulsions was recorded in non-protected mice.

Pentylenetetrazol (PTZ) test

The method has been described previously (Ngo Bum et al. 2001; Schmutz et al., 1990). Clonic seizures were induced in male mice by the i.p. injection of 70 mg/kg PTZ. The protective effect of the plant was recorded in mice treated 1 h before with the decoction. The time of onset of seizures in non-protected mice was also recorded. There were two control groups, one receiving placebo and a positive control group receiving 0.1 mg/kg clonazepam.

Statistical analysis

Two parameters were measured:

i. The protection against NMDA-, STR- and PTZ-induced turning behavior and seizures that was expressed as percentage of animals protected. The Fisher test (two-tail) was used to compare percentage of protected mice in each case.

ii. The time of onset of turning behavior, seizures and the total sleep time. For those three parameters, the mean values of the control group were compared to the mean values of the groups treated with the extract using the Correction for multiple t-test by Bonferroni method.

The ED_{50} values (dose at which 50% of the animals are protected) were calculated using Prism 3.0 software (GraphPad, San Diego, CA).

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Chemicals and Abbreviations

D- (2)- amino-7phosphonoheptanoic acid (D-AP7), Clonazepam (Clonaz), Strychnine (STR), N-methyl-D-aspartate (NMDA), Pentylenetetrazol (PTZ) are all from Sigma chemical USA.

Results

Effect of *Passiflora edulis* on diazepam-induced sleep

The total sleep time induced by diazepam increased significantly from 31 ± 11 min in the control group to 77.6 ± 15 and 78.3 ± 16 min in the groups treated with extract at the doses of 132.5 and 1325 mg/kg respectively (Figure 1). The sleep time of control group was multiply by 2 by extract from dose 132.5 mg/kg.

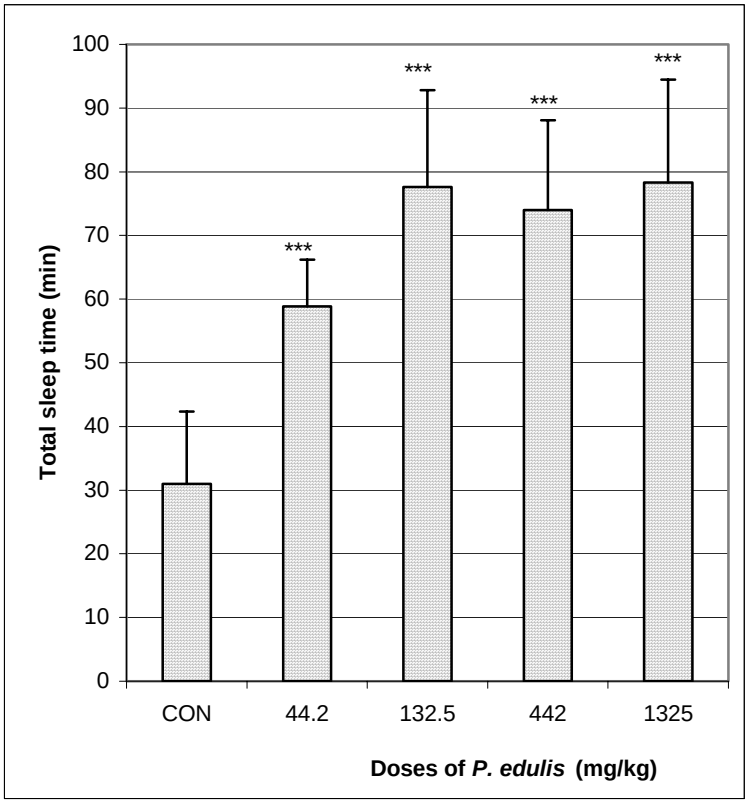

Figure 1: Effect of *Passiflora edulis* on diazepam-induced sleep in mice.
The figure represents the total sleep time (min) induced by diazepam (50 mg/kg, i.p.) in the presence of different treatments in mice. The total sleep time increased with the dose of *Passiflora edulis*. It is expressed as means + SD, n = 6 per dose, *** = $p < 0.001$ (Correction for multiple t-test by Bonferroni method). CON = distilled water.

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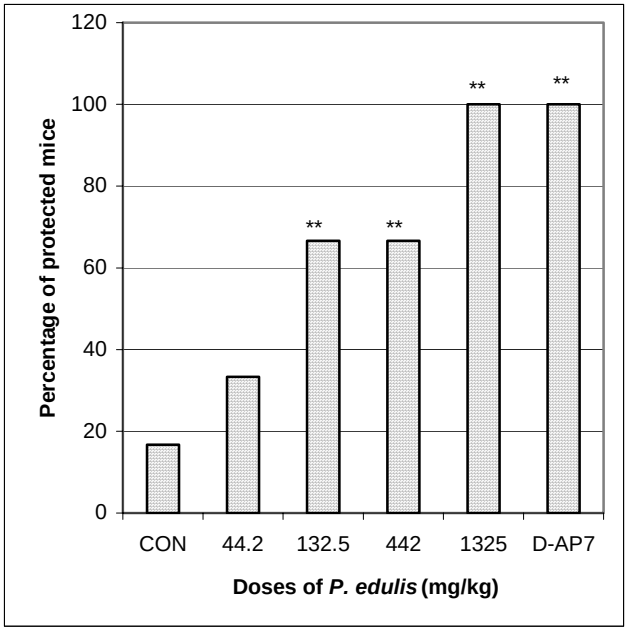

Figure 2a: **Effect of *Passiflora edulis* on NMDA-induced turning behavior in mice.**
Figure 2a shows the percentage of protected animals. *Passiflora edulis* significantly protected mice against NMDA-induced turning behavior in a dose-dependent manner. N = 6 per dose, ** = $p < 0.01$, *** = $p < 0.001$ (Fisher exact test: two tail). CON = distilled water. D-AP7 = D- (2)- amino-7phosphonoheptanoic acid 0.33 ηg/kg

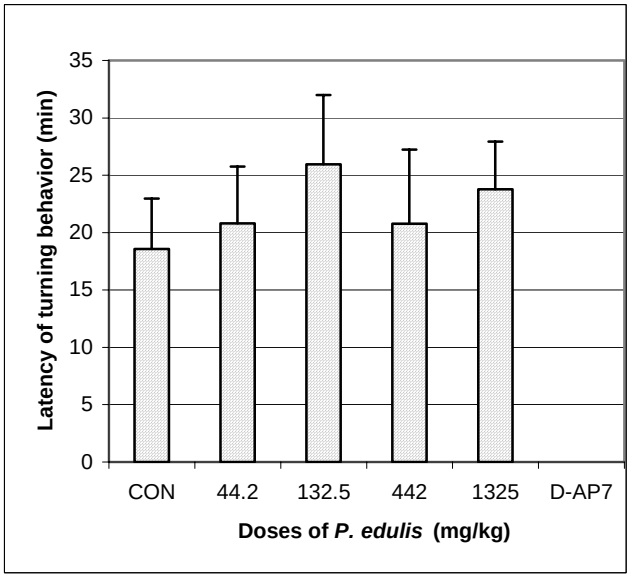

Figure 2b shows the time (min) of the latency of seizures in non-protected animals. *Passiflora edulis* did not provide increase in the time to the onset of the turning behavior in mice. The control group was treated with D- (2)- amino-7phosphonoheptanoic acid 0.33 ηg/kg (D-AP7) or distilled water. N ≥ 2 per dose, * = $p < 0.05$, (Correction for multiple t-test by Bonferroni method). CON = distilled water.

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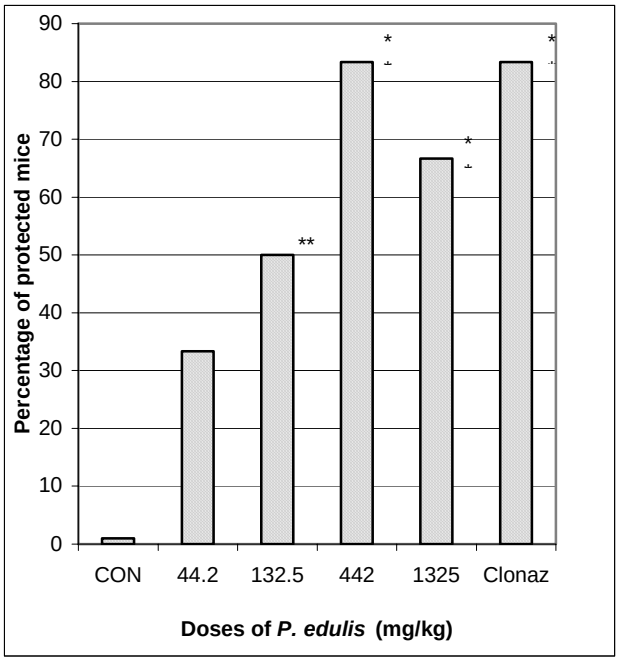

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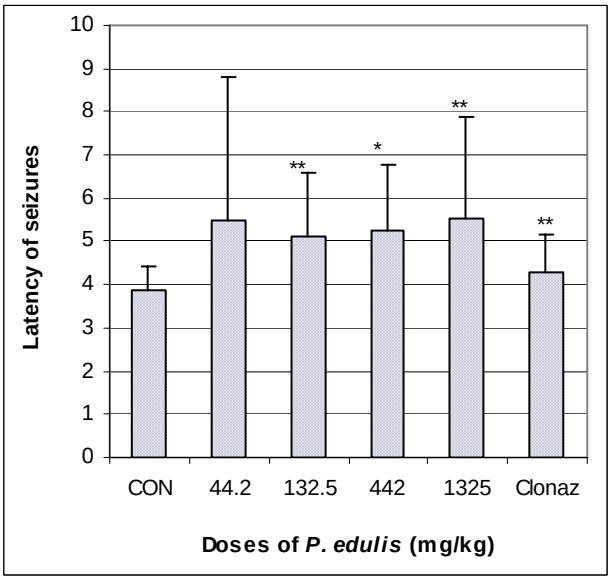

Figure 3: Effect of *Passiflora edulis* on STR-induced tonic seizures and death in mice.
Upper panel A: The effect is displayed by the percentage of mice protected while the lower panel shows the latency of seizures. *Passiflora edulis* significantly protected mice against STR-induced tonic seizures and death. This effect is dose-dependent. N = 6 per dose, ** = $p < 0.01$, *** = $p < 0.001$ (Fisher exact test: two tail). CON = distilled water. Clonaz = clonazepam 3 mg/kg.

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Effect of *Passiflora edulis* on NMDA-induced turning behavior

The extract of *Passiflora edulis* prevented mice from turning. This effect was dose-dependent: 33.3% and 66.6% of the animals were protected at the doses of 44.2 and 132.5 mg/kg i.p., respectively. A dose of 1325 mg/kg produced the same (100%) protection as D-AP7 (0,33 ηM), a competitive NMDA antagonist (Figure 2a). *Passiflora edulis* could not delay the time of onset of turning behavior (Figure 2b).

Effect of *Passiflora edulis* on STR-induced seizures and death

Passiflora edulis extract dose-dependently and significantly increased the number of mice protected against STR-induced seizures and death. The percentage of protection were (50 and 83.3%) at doses of 132.5 and 442 mg/kg i.p. respectively. The percentage of mice protected at a dose of 442 mg/kg of the extract was the same provided by clonazepam, a known anticonvulsant (Figure 3a). The extract significantly delayed the onset of tonic convulsions (Figure 3b).

Effect of *Passiflora edulis* on PTZ-induced seizures

The decoction of *Passiflora edulis* had very small effect against clonic seizures induced by PTZ. At a dose of 1325 mg/kg i. p., *Passiflora edulis* protected only 33.3% of mice (Figure 4).

Discussion and Conclusion

The extract of *Passiflora edulis* potentiated the total sleeping time induced by diazepam. The total sleep time of the control group (31 min) was multiplied by a factor ≥ than 2 in the presence of the extract of *Passiflora edulis* from dose of 132.5 mg/kg. By potentiating diazepam-induced sleep extract of *Passiflora edulis* seem to possess sleep-inducing properties (Guillemain and Tetau, 1980; Rakotonirina et al., 2001). The extract of *Passiflora edulis* also antagonised chemically- induced seizures in mice. *Passiflora edulis* significantly protected mice against STR -induced seizures in mice, with an ED_{50} of 320 mg/kg i.p. Turning behavior induced by NMDA in mice was dose-dependently antagonised by the extract of *Passiflora edulis* with an ED_{50} equal to 300 mg/kg. But the effect on PTZ-induced seizures was not significant. The inhibition by the extract of *Passiflora edulis* of STR-induced seizures and NMDA-induced turning behavior suggests the involvement of glycine and NMDA receptors (Findlay et al., 2002; Ngo Bum et al., 2001) and the presence of anticonvulsant properties (Fisher, 1989, Rogawski, 1992). As PTZ have been shown to interact with the GABA neurotransmitter (De Deyn et al., 1992; Doctor et al., 1982; Löscher and Schmidt, 1988), the lack of effect on PTZ- induced seizures suggests that the extract of *Passiflora edulis* might have no effect on the GABA-ergic neurotransmission. There is a link in the diazepam increasing activity and strychnine- induced seizures produced by *Passiflora edulis.* CNS effect of the decoction in sedation could be interacting with benzodiazepine receptor and not GABA, (Wolfman, et al.1994) likewise, inhibitory effect on STR-induced seizure is probably on glycine and not GABA, while NMDA induced turning effect was reduced in dose dependent manner and was mediated through NMDA receptor and blocked by D-AP7. It therefore shows that the decoction

of *Passiflora edulis* brought about these central actions by interacting with either inhibitory glycine or NMDA amino acid neurotransmitters.

In conclusion, the results of this study suggest the presence of sedative and anticonvulsant properties in the extract of *Passiflora edulis* collected at Bertoua (Cameroon). Those sedative and anticonvulsant properties could then explain part of its use in the traditional medicine in Cameroon.

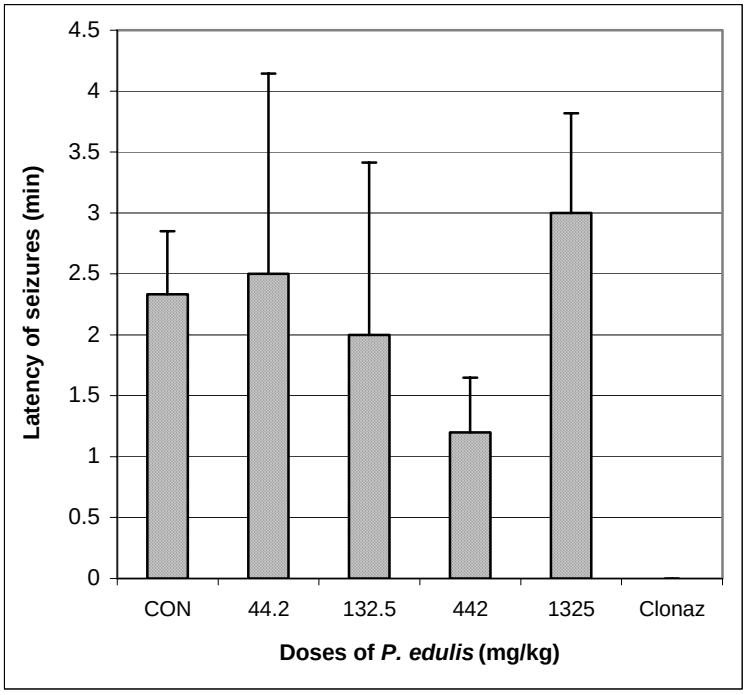

Figure 4: **Effect of *Passiflora edulis* on PTZ-induced clonic seizures in mice.**
Figure 4 shows the percentage of protected animals. *Passiflora edulis* could not protect mice against PTZ-induced clonic seizures. N = 6 per dose, CON = distilled water. Clonaz = clonazepam 0.1 mg/kg.

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