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Effects of the Hydroalcoholic Extract of *Passiflora edulis* on Anxiety Induced by Sub-acute Immobilization Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Author AMRE designed the study and wrote the protocol. Authors KKA and DSSL performed biochemical assays. Authors MOFC, NLD and DSSL performed behavioral tests. Author DT leaded the project and managed statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out to assess the anxiolytic effects of the hydroalcoholic extract of *P. edulis.*
Place and Duration of Study: Animal Physiology Laboratory of the Higher Teachers' Training College, Animal Physiology Laboratory of the Faculty of Sciences , University of Yaoundé I, from November 2017 to August 2018.
Methodology: Anxiety was induced to mice by the sub-acute immobilization stress. After 11 days treatment, behavioural parameters were assessed using Elevated Plus Maze (EPM) and Open

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Field (OF), then biochemical parameters (MDA, GSH, SOD, catalase, GABA, GABA-T and 5-HT) were estimated.

Results: The results show that treatment with *P. edulis* at doses 100 and 200 mg/kg significantly increased open arms entries and time, while reducing closed arms entries and time in the EPM test. Lines crossed as well as passages through the centre and the centre time were significantly increased in the OF test. It is suggested that *P. edulis* would protect against anxiety and this effect probably linked to its ability to fight oxidative stress and counteract hyperexcitability by potentiating the GABA action. The more effective dose, 100 mg/kg significantly ($P<0.01$) increased to 4.44 ± 0.24 µmol/g the activity of GSH. In mice treated with dose 100 mg/kg, the extract induced a significant decrease of three oxidative stress markers including MDA, catalase and SOD to 0.22 ± 0.01 µmol/g, 1.05± 0.15 mmol H_2O_2/min/g; and 19.46±0.00 unit/min/mg respectively when compared to the negative control. Animals treated with *P. edulis* 100 mg/kg presented a significant increase level ($P<0.001$) of GABA and 5-HT up to 4.62 ± 0.28 and µg/g and 31.47 ± 1.58 ng/ml respectively. GABA-T activity was also impacted by the treatment with *P. edulis*, since the value of GABA-T activity of 1.27 ± 0.10 in the negative control significantly ($P<0.001$) decreased to 0.37± 0.00 in the group treated with dose 100 mg/kg.

Conclusion: The beneficial effects of this extract observed in this study justify the empirical use of *P. edulis* in the treatment of head ache and insomnia.

Keywords: Anxiety; Passiflora edulis; immobilisation stress; oxidative stress markers; Neurotransmitters.

1. INTRODUCTION

Psychosis is a chronic recurrent neuropsychiatric disorder affecting the intellectual functioning, behaviour, emotions and quality of life. In Cameroon, as in other developing countries, psychosis is a major public health problem. Although the aetiology is poorly understood, hypo-activity of some neurotransmitter systems including, GABA, dopamine and serotonin, is closely linked to the pathogenesis of psychosis. People with psychosis are more prone to suicide, aggression, drug addiction, cognitive impairment, poverty and increased medical problems, depression, insomnia and anxiety [1]. Anxiety disorders are medical conditions the most striking feature of which is the occurrence of physical or mental symptoms, in the absence of organic brain disease or other psychiatric disorder. Anxiety is a ubiquitous symptom. It is a mental state of turmoil and restlessness, with an indefinable feeling of insecurity, an objectless fear. The prevalence of panic disorders during life varies from country to country from 1.4% to 2.9% [2]. In the general population aged 18 to 65, all anxiety disorders have a prevalence of about 15% over 12 months, about 21% over a lifetime. At the earlier stage they are manifested by spasms, palpitations, sweating, sweaty hands, dry mouth, dizziness, chest tightness. In the long term, complaints are of psychological and this results in impulsiveness, irritability, discouragement, sadness, fatigue, insomnia, sexual inability [1,2]. The inappropriate use of antipsychotics such as benzodiazepines; B-blockers or antidepressants and poor adherence to treatment have favoured the appearance of an addiction phenomenon. The side effects of these drugs can affect all body systems and range from annoying photosensitivity for example, to disabling seizures among others, potentially fatal agranulocytosis and neuroleptic malignant syndrome [3]. The overall functional and quality of life of patients remain poor after treatment and the clinical efficacy of these drugs is largely limited by the adverse effects associated with their use [4]. There is currently a critical need to search for more effective and less toxic agents for the treatment of anxiety disorders. Thus, despite scientific progress in the pharmacological field, the therapeutic use of medicinal plants remains an important source of treatment for psychoses. The (economic) inaccessibility of essential drugs, insufficient health care and socio-cultural habits may justify the use of traditional medicine to treat their pathologies, including psychoses. *Passiflora edulis* (*P. edulis*) is a plant of the Passifloraceae family, empirically used in the region of western Cameroon, more precisely in the Upper NKam subdivision in the treatment of several pathologies including psychosis. Phytochemical studies have shown that this plant contains alkaloids (harmane), flavonoids (vitexol), carbohydrates, essential oil [5]. The present study was undertaken to assess the protective effect of the hydroalkolic extract of the leaves of *P. edulis* against induced-anxiety by sub-acute immobilization stress in mice.

2. MATERIALS AND METHODS

2.1 Plant

The aerial parts of *P. edulis* used in this study were harvested during January 2018 in Bafang town, Upper Nkam Subdivision, West Region (Cameroon). To confirm its identity, a sample of this plant was brought to the National Herbarium of Cameroon (NHC) for comparison with a reference specimen. After identifying the plant material, the leaves of *P. edulis* were removed from the plants and then dried in the shade for a month. Once dried, these leaves were ground in a mill to obtain the green powder used for the preparation of the hydro-alcoholic extract.

2.2 Animals

White mice *Mus musculus* swiss strain of both sexes were used. Animals provided from a colony bred at the animal room of the Faculty of Sciences of the University of Yaoundé I (Cameroon). The environmental parameters of the animal room were as follows: A relative humidity of 50 ± 10%; An average temperature of around 23ºC; A normal cycle of 12 hours light/darkness. Throughout the breeding period, the mice were fed a controlled diet based on a well-known feed and had free access to tap water

2.3 Preparation of the Hydroalcoholic Extract

To prepare the hydro-alcoholic extract of the leaves of *P. edulis*, 735 g of powder were macerated in 6 L of a hydro-alcoholic solution (30% water/ 70% ethanol 97°) for 72 hours. After filtration with Whatmann paper N°3, the macerate was evaporated at 70ºC using a rotary evaporator. The concentrated filtrate was subsequently dried in an oven at 45°C. 80.85 g of dry residue representing the hydroalcoholic extract of *P. edulis* were obtained, i.e. a yield of 11%.

2.4 Evaluation of the Anxiolytic Activity of the Hydroalcoholic Extract of *P. eduli* Leaves on the Stress Induced by Immobilization

2.4.1 Distribution and treatment of animals

Animals were weighed and randomized into 6 homogenous groups (sex and weight) of 6 mice each, then each group underwent a particular treatment: The neutral control group (NC) consisting of naïve mice that received the 10% tween solution; The negative control group (NegC) consisting of mice treated with 10% tween and subjected to sub-acute immobilization stress; The positive control group consisting of animals treated by diazepam at dose 3 mg/kg (Diaz); Three tests groups, each consisting of mice treated by the hydro-alcoholic extract at dose 100, 200 and 400 mg/kg (E100, E200 and E400).

2.4.2 Induction of sub-acute stress by immobilization

Every day at 9 am, each group of animals received a particular treatment, and then subjected to stress. The immobilization stress model used was previously described by bardin and his collaborators in 2009 [6]. For 11 consecutive days, after an initial treatment, for 3 hours, each mouse was individually inserted in a perforated cylindrical bottle to reduce it activity. At day 12 of experiment, all the mice were brought back to their home cages and the level of anxiety assessed by the behavioural tests including the EPM and the OF tests.

2.4.3 Behavioural tests

- **Elevated Plus Maze:** The paradigm used here was similar to those initially described by Lister and his collaborators [7]. After the stress induction period, each animal was individually placed in the centre of the device, facing one open arm and it behaviour observed for 300 s. The parameters noted were as follows: Open arms entries and time; Closed arms entries and time.
- **Open field:** The method used here was initially described by Belzung and collaborators in 1999 [8]. After the EPM test, each animal was individually placed in one of the corners of the open field, then the behaviour observed for a cut off time of 300 S. The parameters noted were as follows: Crossings, passage in the centre, time spent in the centre and the periphery of the paradigm.

2.4.4 Animal sacrifice and sample collection

At the end of behavioural experiments, animals were anesthetized with ether and sacrificed by decapitation. The brains were removed and divided in two halves. The first half was ground separately in a ceramic mortar, homogenized in 2 mL of Tris-HCl buffer (50 mM; pH=7.4) and

centrifuged at 4000 rpm at 4ºC for 25 min. The other half was ground separately, homogenized in 2 mL of methanol and centrifuged at 4000 rpm at 4ºC for 25 minutes. the supernatant was collected and stored in the freezer at -20ºC, then used in the assessment of some parameters of the central nervous system (GABA, GABAT, 5-HT) and oxidative stress markers (SOD, CATALASE, GSH, MDA).

2.4.5 Assay of some oxidative stress parameters

- **Superoxyde dismutase (SOD):** The superoxide dismutase activity was determined by the method described by Misra and Fridovich in 1972 [9].
- **Catalase:** The method described according to Cohen and colleagues in 1970 was used in the determination of Catalase Activity [9].
- **Reduced glutathione:** The level of reduced glutathione (GSH) in the samples was determined by the method described by Jollow and collaborators in 1974 [9].
- **Malondialdehyde:** The method initially described by Wilbur and colleagues in 1949 was used to determine the level of MDA in the samples [10].

2.4.6 Neurotransmitter assays

- **GABA:** The amount of gamma aminobutyric acid (GABA) in the samples was evaluated by the colorimetric assay technique for mouse brain homogenates as described by Lowe and colleagues in 1958 [11].
- **GABA-transaminase:** The activity of GABA-transaminase (GABA-T) was evaluated by the colorimetric assay method of Nayak and Chatterjee in 2001 [12].
- **Serotonin:** The method which has been used is that described by Schlumpf and colleagues in 1974 [13].

3. RESULTS

3.1 Effects of *P. edulis* on the Sub-acute Anxious Behaviour of Mice Induced by Eleven Days of Stress by Immobilization

3.1.1 Anxiolytic properties assessed by the EPM test

- **Effects of *P. edulis* on the open and closed arms entries:** The average open arms entries of 10.2 ± 0.58 in the neutral control significantly ($p < 0.001$) decreased to 1.4 ± 0.24 in the negative control. In groups the groups treated with the extract of *P. edulis* at doses 100 and 200 mg/kg, the plant induced a significant ($p < 0.01$) increase of open arms entries to 6.2 ± 0.83 and 6.6 ± 0.89 respectively when compared to the negative control group. Mice of the neutral control and negative controls group visited the closed arms 10.08 ± 1.2 and 16.6 ± 0.92 respectively. In comparison to the negative control group, treatment with dose 100 mg/kg of *P. edulis* resulted in a significant ($p < 0.001$) decrease up to 4.6 ± 0.44 of the closed arms entries (Fig. 1).
- **Effects of *P. edulis* on the time spent in the open and closed arms of the EPM:** The mean time spent in the open arms, was 19.6 ± 0.67 and 114 ± 3.74 in the negative control and neutral control groups respectively. The treatment with increasing doses of the extract of *P. edulis* resulted in a significant ($p < 0.001$) increase of the open arms time to 154 ± 3.94 and 205 ± 8.76 in groups treated with doses 100 and 200 mg/kg of the hydroalcoholic extract of *P. edulis* respectively. Conversely it was observed a significant ($p < 0.001$) decrease of the closed arms entries to 73 ± 1.37 and to 55 ± 8.45 in the groups treated with *P. edulis* 100 and 200 mg/kg when compared to the negative control group (Fig. 2).

3.1.2 Anxiolytic properties assessed by the open field test

- **Effects of *P. edulis* on the crossed lines and the passages in the centre:** The average number of lines crossed of 150 ± 1.41 in the neutral control group, significantly ($p < 0.01$) decreased to 46.4 ± 5.58 in the negative control group. In comparison to the negative control group, a significant ($p < 0.001$) increase to 260.4 ± 3.50 was observed in groups treated by dose 100 mg/kg. Similarly, diazepam induced a significant ($p < 0.001$) increase in this number when compared to the negative control. The mean number of passages through the centre of the open field, which was 1.4 ± 0.24 in the neutral control group, significantly ($p < 0.01$) decreased in the negative control group. On the other hand, the number of visits to the centre increased with all the doses of *P. edulis* groups, more significantly (p

<0.001) at dose 100 mg/kg when compared to the negative control group (Fig. 3).

- **Effects of *P. edulis* on the time spent in the centre and the periphery:** Mice of neutral control group spent about 2.4 s in the central part of the arena and this time is completely annulled in mice of the negative control group. Treatment with various doses of the hydroalcoholic extract of *P. edulis* induced a significant increased of this time, more marked at dose 100 mg/kg (p <0.001). In the mice of the neutral control group, the average time spent in the periphery of the device was 157 ± 24.54 s. In animals of the negative control group, this value significantly increased (p <0.01) to 300 ± 12 and then significantly (p <0.01) decreased to 174.4 ± 3, 28 in the group treated with *P. edulis* at dose 100 mg/kg in comparison to the negative control group (Fig. 4).

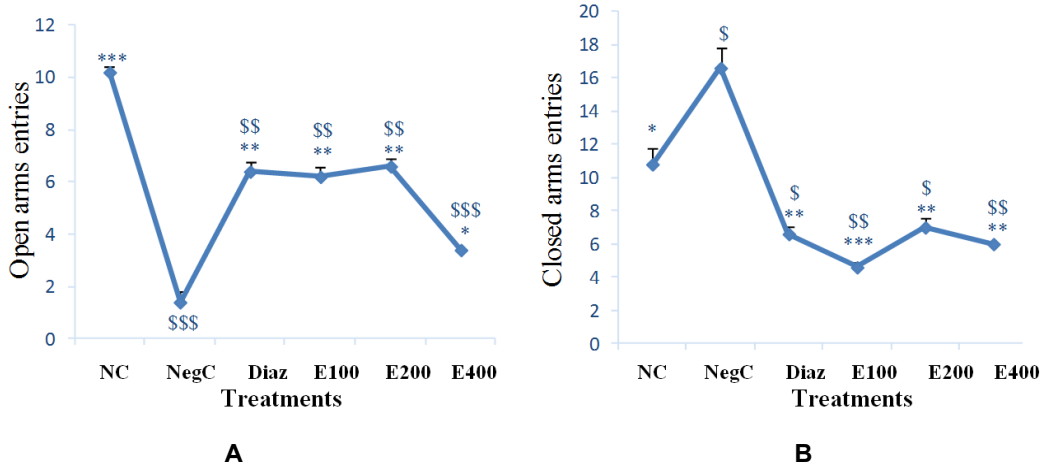

Fig. 1. Effects of *P. edulis* on the open (A) and closed (B) arms entries

*Each point represents the mean ± SEM of the group; n = 6; $ p <0.05; $$ p <0.01; $$$ p <0.001 significant difference compared to the neutral control and * p <0.05; ** p <0.01; *** p <0.001 significant difference compared to the negative control. NC: Neutral control; NegC: Negative control; DIAZ: Positive control treated with diazepam (3 mg/kg); E100, E200, E400: Test groups treated with different doses of the hydroalcoholic extract of P. edulis (100, 200 and 400 mg/kg)*

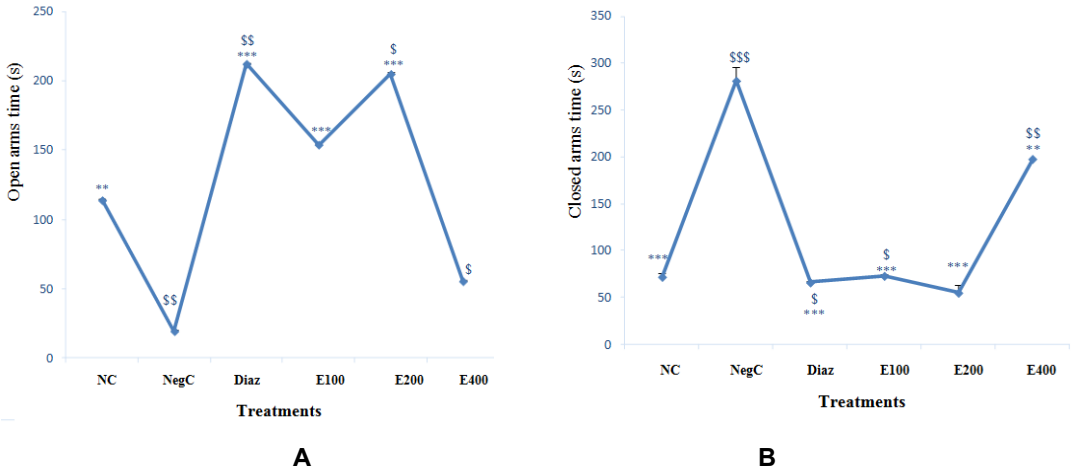

Fig. 2. Effects of *P. edulis* on the time spent in the open (A) and closed (B) arms of the EPM

*Each point represents the mean ± SEM of each group, n = 6; $ p <0.05; $$ p <0.01; $$$ p <0.001 significant difference with the neutral control and ** p <0.01; *** p <0.001 significant difference compared to the negative control. NC: Neutral control; NegC: Negative control; DIAZ: Positive control treated with diazepam (3 mg/kg). E100, E200, E400: Test groups treated with different doses (100, 200 and 400 mg/kg) of the hydroalcoholic extract of P. edulis*

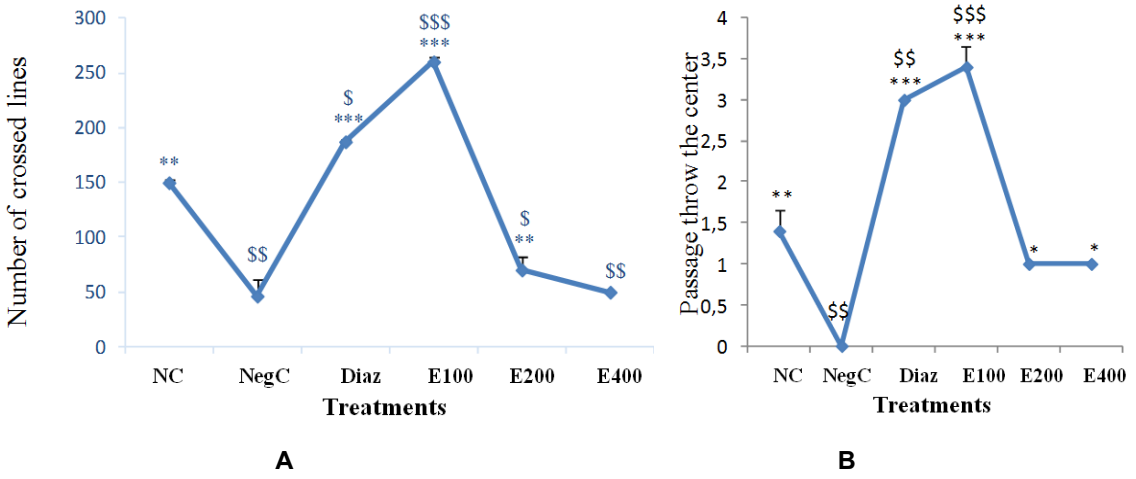

Fig. 3. Effects of *P. edulis* on the number of lines crossed (A) and the number of passages in the centre (B) of the open field

*Each point represents the mean ± SEM of each group, n = 6; $ p <0.05; $$ p <0.01; $$$ p <0.001; significant difference compared to the neutral control and * p <0.05; ** p <0.01; *** p <0.001 significant difference compared to the negative control. NC: Neutral control; NegC: Negative control; DIAZ: Positive control treated with diazepam (3 mg/kg); E100, E200, E400: Test groups treated at different doses (100, 200 and 400 mg/kg) of the hydro-alcoholic extract of P. Edulis*

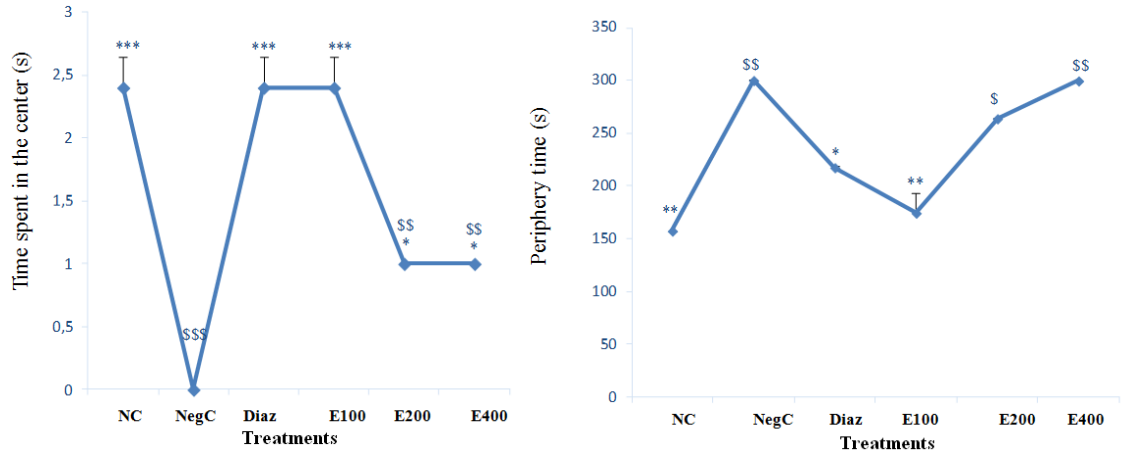

Fig. 4. Effects of *P. edulis* on the time spent in the centre (A) and the periphery (B) of the open field

*Each bar represents the mean ± SEM of each group, n = 6; $$ p <0.01; $$$ p <0.001 significant difference with the neutral control group and * p <0.05; ** p <0.01; *** p <0.001 significant difference when compared to the negative control. NC: Neutral control; NegC: Negative control; DIAZ: Positive control treated with diazepam (3 mg/kg); E100, E200, E400: Test groups treated at different doses (100, 200, and 400) with the hydro-alcoholic extract of P. edulis*

3.1.3 Effects of *P. edulis* on some oxidative stress markers

Table 1 summarizes the effects of *P. edulis* extract on the concentration of malondialdehyde (MDA), reduced glutathione (GSH), and the activity of superoxide dismutase (SOD) and catalase. It appears that the concentration of MDA 0.15 ± 0.02 µmol/g of tissue in the neutral control significantly (p <0.001) increased up to 0.45 ± 0.03 µmol/g of tissue in the negative control. At all doses used, *P. edulis* induced a

significant decrease in this concentration, with the maximum value of 0.22 ± 0.00 µmol/g of tissue ($p<0.001$), achieved with dose 100 mg/kg. The concentration of reduced glutathione of 4.44 ± 0.24 µmol/g of tissue in the neutral control group significantly ($p <0.001$) decreased by 62.16% in the negative control group. At dose 100 mg/kg, *P. edulis* significantly ($p <0.01$) increased this concentration up to 3.90 ± 0.21 µmol/g, an increase of 56.92%. Diazepam, the reference drug, also induces a significant increase ($p <0.01$) in this concentration up to 3.43 ± 0.18 µmol/g (51.02%), when compared to the negative control group. The activities exhibited by catalase and SOD were respectively 0.39 ± 0.32 mmol H_2O_2/min/g and 19.44 ± 0.03 unit/min/mg of tissue in the neutral control group. In the negative control group, catalase and SOD activities increased to 2, 36 ± 0.15 mmol H_2O_2/min/g and 19.67 ± 0.0 unit/min/mg of tissue. At all doses of extract used, *P. edulis* induced a significant increase ($p <0.01$) in the activity of these enzymes in mice treated at doses 100 and 200 mg/kg.

3.2 Effects of *P. edulis* on Some Biochemical Parameters (GABA, GABA-T and Serotonin)

Table 2 summarizes the effects of *P. edulis* on the concentrations of GABA, serotonin and the activity of GABA-transaminase in the limbic system of mice brain. It appears that the concentration of GABA significantly ($p <0.01$) decreased up to 60.59% in the negative control group when compared to the neutral control, thus varying from 3.41 ± 1.21 µg/g of tissue to 1.35 ± 0.29 µg/g of tissue. *P. edulis* induced a significant ($p <0.01$) increase of GABA concentration to 4.6 ± 0.28, 4.2 ± 0.33 and 3.44 ± 0.28 µg/g of tissue at doses 100, 200 and 400 mg/kg respectively. Serotonin concentrations were 5.96 ± 1.48 ng/ml of tissue in the negative control group and 20.95±1.69 ng/ml in the neutral control group. Treatment of different groups of mice by the extract of *P. edulis* resulted in a significant ($p <0.01$) increase up to 31.47 ± 1.58 ng/ml and 23.56 ± 1.42 ng/ml of the brain serotonin level at doses of 100 and 200 mg/kg

Table 1. Effects of *P. edulis* on some markers of oxidative stress

Treatments Dose (mg/kg)		Oxydative stress parameters			
		Reduced glutathion (µmol/g)	MDA (µmol/g)	Catalase activity ($mmolH_2O_2$/min/g)	SOD activity (unit/min/mg)
NC	-	$4.44\pm 0.24^{***}$	$0.15 \pm 0.02^{**}$	$0.39\pm 0.32^{**}$	$19.44\pm0.03^{**}$
NegC	-	$1.68 \pm 0.03^{\$\$\$}$	$0.45 \pm 0.03^{\$\$\$}$	$2.36 \pm 0.15^{\$\$\$}$	$19.67\pm0.01^{\$\$\$}$
Diaz	3	$3.44\pm 0.18^{\$***}$	$0.35 \pm 0.02^{\$\$*}$	$2.22 \pm 0.17^{\$\$\$}$	$19.67\pm0.00^{\$\$\$**}$
E100	100	$4.44 \pm 0.24^{\$**}$	$0.22 \pm 0.01^{\$**}$	$1.05\pm 0.15^{\$***}$	$19.46\pm0.00^{***}$
E200	200	$3.36 \pm 0.22^{\$**}$	$0.27 \pm 0.03^{\$**}$	$1.49 \pm 0.10^{\$\$**}$	$19.63\pm0.01^{\$\$*}$
E400	400	$2.60 \pm 0.20^{\$\$*}$	$0.36 \pm 0.02^{\$\$*}$	$1.84\pm 0.19^{\$\$*}$	$19.67\pm0.00^{\$\$\$}$

*Each value represents the mean ± SEM of each group, n = 6. * p <0.05; ** p <0.01; *** p <0.001; significant difference compared to the negative control and $ p <0.05; $$ p <0.01; $$$ p <0.001 significant difference compared to the neutral control. NC: Neutral control NegC: Negative control; E100, E200, E400: Test groups treated with different doses (100, 200 and 400 mg/kg) of the hydroalcoholic extract of P. edulis; Diaz: Positive control treated with diazepam (3 mg/kg)*

Table 2. Effects of *P. edulis* on GABA, serotonin concentrations and on GABA-T activity

Treatments Dose (mg/kg)		Neurotransmitters		
		GABA (µg/g)	GABA-T activity (unit/mg/proteins)	Serotonin (ng/ml)
NC	-	$3.41 \pm 1.21^{**}$	$0.79 \pm 0.01^{**}$	$20.95\pm 1.69^{**}$
NegC	-	$1.35 \pm 0.29^{\$\$}$	1.27 ± 0.10	$5.96\pm 1.48^{\$\$}$
Diaz	3	$3.59 \pm 0.27^{**}$	$1.23 \pm 0.07^{\$***}$	$13.29 \pm 2.10^{\$*}$
E100	100	$4.62 \pm 0.28^{\$***}$	$0.37\pm 0.00^{\$***}$	$31.47 \pm 1.58^{\$\$**}$
E200	200	$4.20 \pm 0.33^{\$\$**}$	$0.74 \pm 0.00^{**}$	$23.40\pm 1.42^{**}$
E400	400	$3.44 \pm 0.28^{**}$	$1.39 \pm 0.07^{\$}$	$8.28\pm 1.80^{\$\$}$

*Each value represents the mean ± SEM in each group, n =6. $ P <0.05; $$ p <0.01; significant difference compared to the neutral control group and * p <0.05; ** p <0.01; *** p <0.001 significant difference compared to the negative control group. NC: Neutral control NegC: Negative control; E100, E200, E400: Test groups treated at different doses (100, 200 and 400 mg/kg) of the hydroalcoholic extract; Diaz: Positive control treated with diazepam (3 mg/kg)*

respectively. The activity of GABA-T of 0.79 ± 0.01 unit/mg of proteins in the neutral control group significantly ($p < 0.01$) increased to 1.27 ± 0.10 unit/mg of proteins in the negative control group. The hydroalcoholic extract of *P. edulis* significantly ($p < 0.001$) inhibited this activity for about 70.87% in comparison to the negative control group.

4. DISCUSSION

The objective of the present study was to evaluate the effects of the hydroalcoholic extract of the leaves of *P. edulis* on anxiety induced by sub-acute immobilization stress. Sub-acute immobilization stress is known to produce both psychological and physical stress, responsible of a wide range of behavioural and physiological changes such as reduced locomotor activity, anxiety, stress hormones (corticosterone) secretion and oxidative stress [14,15]. In this work, after 11 days immobilization stress, animals' behaviour was assessed on the EPM and OF paradigms. Initially described by Pillow and colleagues, the EPM is a standard device used to assess the anxiolytic and anxiogenic effects of substances and plants in rodents [16]. In the EPM test, open arms entries increased in the presence of diazepam as well as extract of *P. edulis* at doses 100 and 200 mg/kg. Conversely, both extract (100 and 200 mg/kg) as well as diazepam reduced the closed arms time. According to previous works, an increase in mice open arms activity refers to a decrease in anxiety, and a decrease of so-called behavioural parameters in closed arms reflects a reduction in stress/anxiety [17,18]. This result suggests that the extract of *P. edulis* has anxiolytic properties similar to those of diazepam. The anxiolytic activity observed in the EPM was confirmed by the results obtained through the OF, a device which provokes in rodents a reaction of fear to a new environment, the isolation of the social environment or finally a motivation to restore the contact with member of the specie [19,20]. In this test like diazepam, the extract of *P. edulis* at dose 100 mg/kg increases the number of lines crossed, and the time spent in the centre of the paradigm, while reducing the time spent on the periphery. The decrease in time spent at the periphery refers to a decrease in anxiety. However, an increase in the number of crossings, and the time spent in the centre of the OF is a manifestation of the increase in locomotor activity and level of mice exploration, suggesting that the alcoholic extract of *P. edulis* has anxiolytic properties similar to those of diazepam, these effects could be mediated either by GABAergic transmission, or by serotonergic transmission [7,21]. The OF test seems to be sensitive only to conventional benzodiazepines and 5-HT1A receptor agonists, thus the anxiolytic effects of *P. edulis* could be justified by the binding of its constituents to GABAA and serotonin receptors [22,23]. In order to determine the molecular mechanisms underlying the presence of the anxiolytic effects as assumed above, the concentration of GABA in different groups of mice were measured, since pathological anxiety is due to the imbalance between excitation and inhibition in the neural circuits of the limbic system [24,25]. It was observed that, sub-acute immobilization stress induced a significant drop in the concentration of GABA in the limbic system of normal mice. Long term stress is generally known to increase cortisol level in the brain, this in turn is responsible of the inhibition of the GABAergic neurons modulation, and therefore in the drop in the concentration of GABA [26]. An increase of GABA concentration by *P. edulis* (100, 200 and 400 mg/kg) in mice subjected to immobilization stress suggests that the extract would interact with GABAergic neurotransmission specifically on its metabolism. Zhang and his team in 2017 demonstrated that all substances that suppress stress-induced anxiety act by GABAergic neurotransmission, therefore our results are in line with the work of Zhang [25]. In addition, the inhibition of GABA-T activity by the extract in part justifies the increase of GABA concentration, responsible of the anxiolytic effects. Since chronic stress is associated with an increase in GABA-T activity, following an alteration of its metabolism by oxidative stress, these results assume that the extract of *P. edulis* is neuroprotective [27]. The hydroalcoholic extract of *P. edulis* at doses 100 and 200 mg/kg also caused an increase of serotonin concentration in the limbic system. These results, like those previously obtained by Sharp, contribute to the anxiolytic properties of *P. edulis*. Indeed, a disturbance in the modulation of the response to sub-acute stress by serotonin is expressed by anxiety, but any substance that corrects this dysfunction would induce anxiolytic effects [14]. Knowing that antidepressant drugs suppress anxiety induced by chronic stress by inhibiting either the reuptake of serotonin or the action of monoamine oxidases, the anxiolytic effect of *P. edulis* extract could also be explained by an increase concentration of biogenic amines (particularly serotonin) by mechanisms involving their reuptake or hydrolysis [28]. Oxidative stress

is one of the pathophysiological mechanisms by which sub-acute or chronic stress initiates neurochemical, behavioural and hormonal changes. Inhibiting oxidative stress would go a long way in healing anxiety. Still with the aim of searching *P. edulis* action mechanism against anxiety, its impact on sub-acute/chronic stress has been determined. In mice treated with tween 80, sub-acute immobilisation stress resulted in oxidative stress marked by an increase of malondialdehyde (MDA) concentration and a decrease of reduced glutathione (GSH) concentration. These observations are in agreement with those of McEwen who showed that chronic stress leads to oxidative stress resulting in the same variation of brain concentrations of MDA and GSH [29]. In fact, chronic stress is associated with an increase in the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme that generates reactive oxygen species (ROS) or free radicals in the mitochondrial chain. These ROS are at the origin of the lipid peroxidation generating MDA and the reduction of GSH levels due to its use for the neutralization of free radicals under the action of glutathione peroxidase [27]. The work of Machawal et al. (2014) point out that free radicals play an important role in the neurotoxicity and hyperexcitability of the amygdale observed during chronic stress. The administration of the hydroalcoholic extract of *P.edulis* at all doses corrected the increase of MDA level and the decrease in the level of GSH. These antioxidant properties are supported by maintaining the activity of catalase and SOD at a very low level, and would suggest a strong capacity of this extract to neutralize free radicals, because similar studies have demonstrated that a preservation of SOD and catalase activity is associated with a strong capacity to neutralize free radicals [25]. All these results demonstrate that the anxiolytic effects of hydroalcoholic extract of *P. edulis* are due not only to an increase in neurotransmitter levels such as GABA and serotonin in the brain, but also by its ability to protect neurons against oxidative stress during chronic stress, justifying it effectiveness compared to diazepam.

5. CONCLUSION

The main objective of the present study was the assessment of the anxiolytic and sedative effects of the hydro-alcoholic extract of the leaves of *P. edulis* in white mouse *Mus musculus swiss.* The results showed that *P. edulis* has anxiolytic properties at low doses and sedative properties at higher doses in mice. The anxiolytic effects of *P. edulis* are probably due to their inhibitory action of GABA Transaminase inducing an increase in GABA in the limbic system. These effects could also be explained by the ability of the extract to significantly increase the levels of reduced glutathione and the activity of superoxide dismutase which are antioxidants playing a neuroprotective role. In addition, *P. edulis* significantly reduces the malondialdehyde levels resulting from the increase in lipid peroxidation in the limbic system of mice facing stressful situations. These beneficial properties of the extract observed in the present study would therefore justify a study for the development of a phytomedicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal handling procedures were carried out in accordance with the National Ethics Guide (FWA-IRB00001954).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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