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# Preliminary phytochemical screening, acute toxicity and effect of *Albuca amoena* extracts on the central nervous system

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**ABSTRACT**

**Introduction:** *Albuca amoena* is a Moroccan-Algerian endemic medicinal plant with various implications. The aim of this study is to identify phytochemical compounds of the plant, check its acute toxicity, and test its anti-depressive, anxiolytic, and analgesic effects on the central nervous system (CNS).

**Methods:** The estimation of chemical compounds was carried out according to coloring and precipitation reactions. The Organization of Economic Cooperation and Development guidelines 423 and 402 made it possible to verify the acute toxicity of the plant orally and dermally. The sedative activity was performed according to 4 tests: rotarod, hole-board, traction, and chimney tests. The anti-depressive, anxiolytic, and analgesic effects were evaluated by forced swimming, light/dark, and writhing tests, respectively.

**Results:** The phytochemical analysis showed that A. amoena contained a mixture of phytochemical compounds like terpenes, alkaloids, and polyphenols. According to the acute toxicity tests, the lethal dose of 50% (LD50) of A. amoena hydroalcoholic extract was between 300 and 2000 mg/kg orally and higher than 2000 mg/kg dermally. Moreover, the result of the behaviour tests of sedative and analgesic activities revealed that A. amoena hydroalcoholic extract exerted positive effects on the CNS.

**Conclusion:** These results show the anti-depressive, anxiolytic, and analgesic effects of the bioactive substances present in A. amoena on the CNS and provide access to further investigations to highlight the main compounds of this plant and their mechanisms of actions.

*Implication for health policy/practice/research/medical education:*
Extracts of *A. amoena* showed remarkable anti-depressive, anxiolytic, and analgesic effects on CNS and did not reveal toxicity. *A. amoena* may have significant implications for the future development of anti-depressive, anxiolytic, and analgesic drugs targeting CNS related pathologies.

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## Introduction

The sedative action of a substance can be defined as its ability to alter the level of vigilance, psychomotor strength, and nociceptive impulses to the individual to which it is administered (1). The most commonly used sedative drugs by humans belong to the benzodiazepine family. They are alkaloids known for their therapeutic virtues, mainly their effects on the central nervous system (CNS), such as anxiolytic, analgesic, muscle relaxant, and sedative properties (2).

Currently, several investigations have shown the effects of natural compounds, including those isolated from medicinal plants, on the CNS and its related pathologies. In North Africa, bulbous plants are traditionally used

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either for the prevention or curative treatment of several diseases (3). *Albuca amoena* (Batt.) J.C.Manning & Goldblatt. is endemic to neighboring Moroccan areas and the North-Western Algerian Sahara. It grows in sandy and stony desert pastures (4,5). *A. amoena* has been used in traditional medicine to treat melanoma and various dermal infections (6). However, to the best of our knowledge, there is no pharmacological study about this species on the CNS. Phytochemical analysis revealed that *A. amoena* contained alkaloids, particularly Colchicaceae (5). Therefore, the aim of the present study consists in performing phytochemical screening, evaluating acute toxicity and testing anti-depressive, anxiolytic, and analgesic effects on CNS about this plant.

## Materials and Methods

### Plant materials and extraction

The plant was collected from Boudnib, Morocco, in March 2019. The plant was identified by Prof. El Alaoui Fariss, and a specimen was deposited at the herbarium of the Scientific Institute of Rabat, Morocco (Vouchers Specimen: RAB108289). The aerial parts (leaf, flower, stem, fruit, and seed) were separated from the bulbous part, dried at room temperature, and used for extraction and phytochemical screening.

Briefly, 100 g of the plant was macerated in 1 L of a hydroalcoholic mixture (1V ethanol: 1V distilled water) for 24 hours three times. The obtained filtrates were evaporated in a vacuum rotary evaporator under reduced pressure at 50°C. The obtained extracts from aerial parts (AP) and bulbous part (BP) of the plant gave dense chestnut extracts with yields of 17.33% and 35.86%, respectively, which stored at 4°C.

### Phytochemical screening

The qualitative phytochemical screening of some bioactive compounds (Mucilage, Oses, holosids, reducing sugars, lipids, alkaloids, polyphenols, flavonoids, tannins, coumarins, anthracenosids, terpenoids, sterols, triterpenes, carotenoids, iridoids, and saponins) was carried out on the vegetable powder of the two parts of the plant (aerial and bulbous) based on conventional methods (7-10).

### Animals

Adult Swiss mice (20-30 g) and Wistar rats (200-300 g) were used in the study. The animals were maintained in the animal center at the Faculty of Medicine and Pharmacy, University Mohammed V, Rabat. The animals were kept in standard environmental conditions in collective cages (temperature 23 ± 3°C and 12/12 hours light/dark cycle) and fed with a standard rodent diet and water *ad libitum* (11).

### Acute oral toxicity

This test was performed using the method described by the Organization for Economic Co-operation and Development (OECD) Guideline 423 (12). Briefly, female mice, fasted 4 hours before, were divided into five groups of three animals each. The control group received normal saline and the test groups were treated with AP and BP extracts at the doses of 300 and 2000 mg/kg, respectively. The animals were observed during the first 6 hours after administration and daily for 14 days for their general behavioral symptoms, mortality, and body weight changes.

### Acute dermal toxicity

This study was carried out using the OECD guideline 402 (13). Seven groups of two rats (one male and one female kept in separate cages) were applied to 10% of the body surface area of animals (shaved the day before) with 200, 1000, and 2000 mg/kg of extracts, respectively. The control group was applied with a vehicle (Vaseline). The animals were observed for 14 days with special attention at first 1, 24, 48, and 72 hours for the presence of any dermal changes.

### Evaluation of sedative activity

The sedative activities of AP and BP extracts were evaluated when the products were administered orally 30 minutes before the behavioral tests (Rotarod, Hole-board, Traction, and Chimney tests). The AP and BP extracts were tested at two therapeutic doses of 100 and 300 mg/kg and compared with bromazepam 20 mg/kg as a reference substance.

#### *Rotarod test*

This test aims to evaluate motor coordination, the level of tiredness and the ability to learn a behavior. This test is carried out by a Rotarod device (Ugo Basile, model 7600) consisting of a horizontal Perspex mast with 50 cm length and 3 cm diameter, providing optimal grip for rodents. Vertical partitions allow delimiting four isolated compartments in order to submit four animals to the test simultaneously. This mast rotates freely around its longitudinal axis due to a direct current motor (14). The mice were submitted to a pre-test 24 hours before the test which consisted of putting them at a speed of 12 turns per minute in order to retain those which would be able to remain at least 60 seconds on the rotating bar. The latency time before the animal's fall was evaluated 30 minutes after the administration of the extracts.

#### *Hole-board test*

This test demonstrates the exploration reaction, a reaction related to both animal's curiosity, and desire to escape. The mice were all maintained in their cages until 30 minutes after the administration of the extracts. The mice were deposited one by one in the center of a board measuring 40 cm × 40 cm and 2.2 cm thick, which had 16 regularly spaced 3 cm diameter holes. It was made of gray Perspex with a matte finish to avoid reflections that could disturb

the behavior of the mice. The count of the number of holes explored was revealed after 5 minutes (15).

### *Traction test*

This method tests the animal's rebalancing reflex. To do this, we seized the mouse through the skin of the back and the tail, presented the wire (1 mm diameter, 15 cm length) with their front legs, released the mouse as soon as the legs grabbed the wire, and counted the time taken by the mouse to bring at least one of the hind legs to touch the wire. A normal mouse performs recovery in less than 5 seconds (16).

### *Chimney test*

This test evaluates the motor coordination, the exploration, and the desire to escape in a mouse facing a new environment. It consists of putting a mouse starting by the head first in a vertical test tube of 30 cm in length, 30 minutes after administration of the extracts. We noted the time taken by the mouse to escape. The mice were sedated if the time exceeded 30 seconds (17).

## Anti-depressive activity

### *Forced swimming test*

This test evaluates the action of antidepressants on the neuromuscular plane. The animal was placed in a cylindrical container 10 cm in diameter and 25 cm deep filled to a height of 12 cm with lukewarm water 60 minutes after administration of the products by the oral route. The time of stillness of the mouse was counted for 6 min. The first 2 minutes show the mouse's desire to get out of it and then it gets tired, comes to a standstill over time, and behaves in desperation (18).

## Anxiolytic activity

### *Light/dark test*

This test makes it easy and quick to assess the animal's exploratory activity, agitation, and mobility (19). The device consisted of two compartments of identical size (20 cm × 10 cm × 14 cm). The first was white and strongly lit with a lamp of 1000 lux, while the second was opaque and dimly lit by a red light of 50 lux. A small opening (5 cm × 5 cm × 5 cm) acted as a door between the two compartments allowing the animal to move freely. At the start of the test, after oral administration of the extracts, the mouse was placed in the dark compartment; its transition between the two dishes and the time spent in the white compartment were measured for 5 minutes (20).

## Analgesic activity

### *Writhing test (Peripheral analgesic activity)*

This test was performed using Koster's method with some modifications (21). It is designed to assess peripheral nociceptive sensitivity of the CNS, mainly muscle nociceptors. The mice (fasting 4 hours before the test) received orally normal saline as a control, aspirin (100 mg/kg) as a reference or the extracts AP and BP (100 mg/kg). The induction of cramps was done 30 minutes after treatment by injecting the mice intraperitoneally with 3% acetic acid at a rate of 3.75 mL per 1 kg of body weight. The number of cramps was counted during continuous observations for 10 minutes starting at 10 minutes after the injection of acetic acid. The total number of frank abdominal twists made it possible to calculate, for each batch treated, the percentage of inhibition of cramps according to the following formula (22):

$$Percent\ inhibition\ (\%) = \left(1 - \frac{Number\ of\ writhings\ in\ treated\ groups}{Number\ of\ writhings\ in\ control\ groups}\right) \times 100$$

## Data analysis

The results were represented on the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA). We took post hoc procedure and $P < 0.05$ was considered statistically significant. The statistical analysis was performed using the software GraphPad Prism 8.

# Results

## Phytochemical screening

The results of the preliminary phytochemical screening of *A. amoena* revealed the presence of different kinds of chemical groups (Table 1).

## Acute toxicity

Acute oral toxicity of AP and BP extracts reduced locomotor activity and caused tremors, vomiting, diarrhea, and then death in mice by increasing the dose administered to 2000

**Table 1.** Phytochemical screening of *Albuca amoena*

| Phytochemical constituents | | | Aerial parts | Bulbous part |
|---|---|---|---|---|
| Primary metabolites | Mucilage | | - | +++ |
| | Oses and holosids | | +++ | +++ |
| | Reducing sugars | | +++ | - |
| | Lipids | | ++ | ++ |
| Secondary metabolites | Alkaloids | Mayer's agent | - | + |
| | | Dragendorff's agent | + | + |
| | Polyphenols | | +++ | - |
| | Flavonoids | Flavonols | - | - |
| | | Flavones | +++ | - |
| | | Flavonones | - | - |
| | Tannins | Catechism | - | - |
| | | Gallic and ellagic | +++ | - |
| | Coumarins | | - | - |
| | Anthracenosids | | - | - |
| | Terpenoids | | +++ | +++ |
| | Sterols and tritepenes | | +++ | - |
| | Carotenoids | | +++ | - |
| | Iridoids | | + | +++ |
| | Saponins | | +++ | +++ |

The presence of chemical constituents is: (+++) important, (++) moderate, (+) slight, and (-) absent.

mg/kg. Thereby, the $LD_{50}$ induced by AP and BP extracts were 300 and 2000 mg/kg, respectively. In contrast, the evaluation of the acute dermal toxicity of the AP and BP extracts affirmed that they did not cause any visible toxicity or death. Therefore, both extracts belong to the fifth category where the $LD_{50}$ was higher than 2000 mg/kg and the product may be harmful in dermal contact.

### Sedative activity

The results obtained from behavioral tests designed to assess sedative activity indicated that AP and BP extracts exerted a significant effect on mice (Table 2). The results of the rotarod test showed that the mice which received a therapeutic dose of 100 mg/kg of the two extracts exceeded 120 seconds on the rotating rod. They retained their motor coordination and their balance in the same way as those in the control group. These mice showed no particular signs of fatigue. The effect of the AP and BP extracts was recorded at a dose of 300 mg/kg with a shoot time of 59 and 80 seconds, respectively. Tiredness, loss of balance, and motor coordination in the mice were clearly visible.

In the hole-board test, the AP extract prevented the mice from exploring a large number of holes. The animals lost their sense of curiosity since at a dose of 100 mg/kg, and they explored an average of 16.8 holes compared to 11.4 holes at the dose of 300 mg/kg. The normal mice explored an average of 26.4 holes. They retained their locomotor activities and sense of curiosity. However, those receiving a dose of 100 mg/kg of the BP extract, slightly decreased the number of holes explored which was on average 20.2 holes. However, at the dose of 300 mg/kg, animals lose their sense of curiosity and explore only 16 holes on average.

For the traction test, it took only a second to straighten the mice on a wire by giving them either normal saline or a dose of 100 mg/kg of both extracts. Indeed, they retained all of their motor coordination and pulling force. At a dose of 300 mg/kg, the AP extract exerted a weak effect on mice as they recovered on average in 1.5 seconds, and at the same dose, the mice receiving the BP extract needed a longer time to get back the hind legs on the wire as it averaged 2.24 seconds.

Moreover, the chimney test revealed that the mice having received AP extract at doses of 100 and 300 mg/kg showed that the latter faced their climb so that they exceeded 30 seconds when leaving the tube and even that they remained motionless in the bottom of the tube; this extract induced muscle weakness and relaxation. In return, the mice receiving only distilled water were able to exit the tube on average in 6 seconds, the same as the doses of 100 and 300 mg/kg of the BP extract.

### Anti-depressive activity

The time of stillness of the control mice was 200 seconds on average. This behavior was very obvious in the mice which received the AP and BP extracts at 300 mg/kg. They stayed still for 205 ± 4.1 and 198 ± 3.91 seconds, respectively (Table 3).

### Anxiolytic activity

During the five minutes of observation, the mice treated with AP at 100 mg/kg were the least anxious and spent 31.85 ± 2.62 seconds in the white compartment compared to 74.37 ± 2.95 seconds in the mice, which received BP at the same dose. The control mice had a stay of 107 ± 2.82 seconds in the white compartment, while the mice treated with diazepam at 3 mg/kg registered 202.5 ± 1.17 seconds (Table 4).

The administration of AP and BP extracts to the mice decreased their agitation and the number of transitions between the two compartments (6.72 ± 2.52 and 7.96 ± 2.38 passages, respectively) compared to the mice of the control group (12.5 ± 3.15 passages).

### Analgesic activity

The injection of acetic acid gave the control batch an average of 46.6 cramps, which made it possible to calculate the percentage of inhibition of pain in the mice that received the therapeutic dose of 100 mg/kg orally of AP, BP, and Aspirin. Aspirin inhibited 58.15% of the nociceptive effect induced by the injection of acetic acid. On the other hand, the analgesic properties of the two extracts AP and BP showed to be superior to that of the reference drug

**Table 2.** Sedative activity of *Albuca amoena* extracts

| Behavioral tests | Control | Bromazepam 20 (mg/kg) | AP | | BP | |
|---|---|---|---|---|---|---|
| | | | 100 (mg/kg) | 300 (mg/kg) | 100 (mg/kg) | 300 (mg/kg) |
| Rota-Rod test<br>Time spent on the stem (s) | > 120 | 2.5 ± 0.9 | > 120 | 59 ± 5.16 | > 120 | 80 ± 51 |
| Hole-board test<br>Number of holes explored | 26.4 ± 12.74 | 0 | 16.8 ± 8.14 | 11.4 ± 6.32 | 20.2 ± 13.9 | 16 ± 7.88 |
| Traction test<br>Recovery time (s) | 1 | 10 ± 0.6 | 1 | 1.5 ± 1.87 | 1 | 2.24 ± 1.73 |
| Chimney<br>Climbing time (s) | 6 | >30 | >30 | >30 | 6 ± 2.6 | 6.2 ± 3.51 |

AP, aerial parts; BP, bulbous part.
All data are mean ± SD (n = 6).

**Table 3.** Forced swimming test of *Albuca amoena* extracts

| Treatment groups | Dose (mg/kg) | Time of stillness (s) |
|---|---|---|
| Control | - | 200 ± 3.62 |
| Fluoxetin | 32 | 100 ± 5.7 |
| AP | 300 | 205 ± 4.1 |
| BP | 300 | 198 ± 3.91 |

AP, aerial parts; BP, bulbous part.
All data are mean ± SD (n = 10).

by reducing the reflex of muscle contractions with an inhibitory percentage of 66.95% and 72.96%, respectively (Table 5).

## Discussion

Here, we highlighted the effects of *A. amoena* extracts on the CNS and explored their toxicities. Plant extracts showed the presence of several chemical families, including alkaloids. The acute oral toxicity of AP and BP extracts manifested the same signs of colchicine overdose, which is toxic and has a narrow therapeutic margin (23). These results are consistent with other works, which demonstrated that the toxicity of *A. amoena* was due to the presence of alkaloids, in particular colchicine (5).

Medicinal plants belonging to the same family, such as *A. amoena,* are also characterized by high oral toxicity and safe use by the dermal route (24). For this reason, *A. amoena* is consumed in low doses. Red squill (*U. maritima and U. noctiflora*) is consumed at low doses and in the case of overdose, intoxication is manifested by vertigo, vomiting, diarrhea, hypertension, and other symptoms that have been recorded (some of them when performing acute oral toxicity) (3,25).

The phytochemical screening of 15 active substances showed the presence of 11 of them in the aerial parts and 7 in bulbs. The important results are the detection of three major families of secondary metabolites, high content terpenes, medium content alkaloids, and polyphenols (only in the aerial part of the plant). Terpenes provide several pharmacological properties. Monoterpenes, triterpenes, and saponins are responsible for anxiolytic, sedative, and analgesic effects (26-28). However, alkaloids are often toxic, exhibit analgesic (morphine, codeine), spasmolytic (tubocurarine, papaverine), and sedative1 (benzodiazepine) properties (29,30). Several investigations revealed that flavonoids (31), lignans (32), and coumarins (33) are among the polyphenols responsible for analgesic effects. Moreover, the sedative effect is induced mainly by tannins (34).

The analysis of the obtained results obtained indicates that two extracts exert a sedative effect on the CNS. They act on the symptoms of anxiety and anguish states, decrease the level of attention and vigilance, contribute to muscle relaxation, inhibit noxious impulses and potentiate the analgesic effect. Serotonin and GABA are the main neurotransmitters involved in the regulation of sedation and analgesia. Intensive stimulation of the GABAergic and serotoninergic receptors induces a depressive action on the CNS (35). Some plants of the Asparagaceae family have sedative effects related to serotoninergic and noradrenergic mechanisms (36,37), by facilitating monoaminergic transmission (38), or even by activating the Shp-2, ErK1/2 and Akt signaling pathways (39). *A. amoena* extracts exert a sedative and analgesic action, probably by agonistic action on GABA and serotonin receptors, facilitating the appearance of their biological effects.

## Conclusion

The topical use of *A. amoena* extracts did not present harmful effects. However, the oral $LD_{50}$ evaluation was limited in the range of 300 and 2000 mg/kg. The sedative

**Table 4.** Light/dark test of *Albuca amoena* extracts

| Treatment groups | Dose (mg/kg) | Time in the light box (s) | Number of transitions |
|---|---|---|---|
| Control | - | 107 ± 2.82 | 12.5 ± 3.15 |
| Diazepam | 3 | 202.5 ± 1.17 | 16.25 ± 2.74 |
| AP | 100 | 31.85 ± 2.62 | 6.72 ± 2.52 |
| BP | 100 | 74.37 ± 2.95 | 7.96 ± 2.38 |

AP, aerial parts; BP, bulbous part.
All data are mean ± SD (n = 6).

**Table 5.** Effects of *Albuca amoena* extracts on acetic acid-induced cramps in mice

| Treatment groups | Dose (mg/kg) | Number of cramps | Percentage of inhibition (%) |
|---|---|---|---|
| Control | - | 46.6 ± 2.71 | - |
| Aspirin | 100 | 19.5 ± 1.73 | 58.15 |
| AP | 100 | 15.4 ± 1.7 | 66.95 |
| BP | 100 | 12.6 ± 2.56 | 72.96 |

AP, aerial parts; BP, bulbous part.
All data are mean ± SD (n = 6).

and analgesic effects of AP and BP extracts on the CNS were observable, attributed to their bioactive compounds containing in this plant. However, further studies should be carried out to determine the chemical composition of this plant and to evaluate their effects on CNS.

## Authors' contributions

RZ performed the tests, analyzed the results, and wrote the manuscript. ME contributed to the achievement of acute toxicity. OE contributed to the performance of the sedative and analgesic tests. AB reviewed the final version. RN guided the phytochemical screening. YC and KA supervised and corrected the study. All authors read and confirmed the final version of paper for publication.

## Conflict of interests

Authors declare that they have no competing interests.

## Ethical considerations

The animal experiment agreed with the principles for the care and use of the animal center at the Faculty of Medicine and Pharmacy, University Mohammed V, Rabat (FMPR0120). The ethical considerations were taken account "the Guide for the Care and Use of Laboratory Animals" prepared by National Academy of Sciences and published by National Institutes of Health.

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