

Preliminary experimental research on acute toxicity of *Vernonia kotschyana* extracts in mice

Studiu experimental preliminar asupra toxicității acute a unor extracte din *Vernonia kotschyana* la șoarece

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Abstract

Vernonia kotschyana Sch. Bip. ex Walp (*Asteraceae*) is used in traditional medicine of Mali to treat gastric diseases and gastro-duodenal ulcer. Three extracts were obtained from vegetal material, represented by roots of *Vernonia kotschyana*: ethyl acetate (V-AE), ethanol (V-E) and water (V-A) extract. The acute toxicity of the three extracts was studied in *Swiss albino* mice. The three extracts were administered orally in single dose in the following sequence: 800, 1600 and 3200 mg/kg body weight. Animals were monitored daily for 14 days. Experimental results showed statistically significant change in water consumption after administration of V-A and V-E extracts. On the studied dose sequence LD50 value could be determined for V-AE extract (2021.09 +/- 1484.2 mg/kg b.w.) that is considered slightly toxic category. V-A and V-E extracts were not found to be toxic on the administered dose sequences. The recorded data encourage further research on vegetal material in terms of phytochemical characterization and the assessment of some potential biological actions.

Rezumat

În medicina tradițională din statul Mali, *Vernonia kotschyana* Sch. Bip. ex Walp (*Asteraceae*) este folosită în vederea tratării afecțiunilor gastrice și ulcerului gastro-duodenal. Din materialul vegetal, reprezentat de rădăcinile speciei *Vernonia kotschyana* au fost obținute trei extracte: în acetat de etil (V-AE), etanol (V-E) și apă (V-A). Toxicitatea acută a celor trei extracte a fost evaluată pe șoareci *Swiss albino*. Cele trei extracte au fost administrate pe cale orală în doză unică, în următoarea secvență: 800, 1600 și 3200 mg/kg corp. Animalele au fost monitorizate zilnic, timp de 14 zile. Rezultatele experimentale au arătat o modificare semnificativă statistic a consumului de apă după administrarea extractelor V-A și V-E. Pentru extractul V-AE s-a putut determina valoarea DL50 (2021,09 +/- 1484,2 mg/kg corp), pentru secvența de doze studiată, care permite încadrarea extractului în categoria de substanțe cu toxicitate mică. Extractele V-A și V-E nu s-au dovedit a fi toxice pentru secvențele de doze administrate. Datele înregistrate încurajează continuarea cercetărilor asupra materialului vegetal, în ceea ce privește caracterizarea fitochimică a acestuia, cât și evaluarea unor potențiale acțiuni biologice.

Introduction

With all the progresses made in the field of synthetic and semisynthetic chemistry, plants still remain an important source of substances with therapeutic potential. Current researches are aimed at the discovery of new

therapeutic alternatives to the classical medication, with safety and increased efficiency, as well as with far less secondary effects.

Vernonia kotschyana Sch. Bip. ex Walp (*Asteraceae*) is an african plant that is used in traditional medicine for the treatment of many

diseases. The roots of the plant are used in Mali to treat digestive dysfunctions (gastro-duodenal ulcer, gastritis, indigestion) and wound healing (Austarheim *et al.*, 2012; Nergard *et al.*, 2004). After washing, grinding and drying, the roots of the plant are sprayed until a very fine powder is obtained. This is given to patients either dry or suspended in warm water three times a day until the symptoms disappear (Inngjerdigen. *et al.*, 2004; Nergard *et al.*, 2004).

Researchers from the *Department of Traditional Medicine* from Mali carried out a preliminary phytochemical screening on the aqueous extract obtained from the roots of this species. The presence of saponosides, catechinic and gallic tannins and polysaccharides (inulin, pectins, mucilages) was highlighted (Germano *et al.*, 1996).

The studies performed on different parts of the plant revealed that it possesses various biological activities: antiulcerous (Ibrahim G. *et al.*, 2009), immune system modulator (Nergard *et al.*, 2005), analgesic and anti-inflammatory (Ibrahim *et al.*, 2009), antibacterial (active against *Salmonella* species and *Staphylococcus aureus*) (Ibrahim, 2012), larvicidal, antiprotozoaric and molluscicide, antioxidant (Diallo *et al.*, 2001).

For the aqueous extract obtained from the roots of the species was conducted an *in vivo* toxicity study on *Artemia salina* Leach salty water crustaceans using the method of Meyer *et al.* (1982) of determination of lethal concentration 50 (LC₅₀) (Meyer *et al.*, 1982). Sanogo *et al.* have set a LC₅₀ higher than threshold value of 1000 µg/mL (Sanogo *et al.*, 1996).

The objective of this study is to investigate the safety-in-use of different plant extracts, having in view that the plant is used in traditional medicine by evaluating the acute toxicity.

Also, Ibrahim *et al.* have conducted an acute toxicity study on ethanol extract obtained from the leaves of the plant that was intravenously administered to *Swiss albino* mice. Apathy and decreased motility of animals were observed as signs of acute intoxication (Ibrahim *et al.*, 2009).

1. Material and Methods

Animals

Swiss albino mice weighting between 25.00-35.00 g were used for the study of acute toxicity. Animals were obtained from *Cantacuzino* Institute, Bucharest. They were acclimatized for 10 days before the experiment with free access to standard food and water *ad libitum*. The animals were housed in polietilene cages in well-ventilated environment with a temperature of 21.00 ± 2.00°C and 12.00 h light-dark cycle. All animal experiments were conducted according to international guidelines regarding bioethics of the study on laboratory animals and the specific rules of the Research Ethics Committee of the „Grigore T. Popa” University of Medicine and Pharmacy Iasi (Directive-UE, 2010).

Plant material

The vegetal material, represented by the roots of *Vernonia kotschyana* Sch. Bip ex Walp. was provided by the *Department of Traditional Medicine*, Bamako, Mali. The identity of plant material has been confirmed by experts from the same department. The vegetal material, dried and pulverized, was stored in cool and dry place, away from light until use. A sample of the plant product is deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, “Grigore T. Popa” U.M.F. Iasi.

Plant extraction

The ethyl acetate (V-AE) and ethanol (V-E) extracts were obtained based on successive extractions with solvents of different polarities as previously described (Vasincu *et al.*, 2014). Following another method of extraction that is similar to the one used in traditional medicine (infusion followed by prolonged maceration) was isolated the aqueous extract (V-A). All the extracts were stored at -20°C until use.

Acute toxicity by oral administration

The mice were randomly divided into three groups (V-A, V-AE, V-E), corresponding to the types of extracts, each group containing three subgroups for the administered dose

sequences. Three hours before the experiment the animals were maintained without food and water. Each treated group received the extracts orally in CMC-Na suspension in single dose in the following sequence: 800, 1600 and 3200 mg/kg body weight.

Observations were made and recorded in the first hour and after two, four and six hours after extracts administration in the first 24 hours. The animals were observed for the following clinical signs and symptoms during 14 days: lethality, motor behavior, reactions to external stimuli, fur and tail aspects, convulsion, tremor, changes in intestinal transit. Animal weights and food and water consumption were recorded daily. On the day 15, all the animals were sacrificed for histological evaluation (Jung E.-Y. et al., 2010; Traesel G.K. et al., 2014).

Determination of lethal dose 50 (DL50) for oral route of administration was performed using the Miller-Tainter method (computerized version). Data interpretation was quantal type (Miller L.C., Tainter M.C., 1944).

The assessment of water and food consumption and body weight was performed using the *t Student* test (GraphPad Prism 6).

2. Results and Discussion

Following oral administration of suspensions of **V-A**, **V-AE** and **V-E** extracts

using the doses mentioned in *Material and Method* section, the following data were obtained.

Table 1 shows a change in water consumption in a dose-dependent manner. The data were statistically significant. During 14 days of experiment, water consumption was fluctuating, with significant deviations from daily average of water consumption. The weight curve and food consumption didn't change during the experiment.

Table 2 shows a change in water consumption, but the statistical parameters do not highlight the significance of consumption. Body weight and food intake didn't change during the experiment.

Table 3 shows a change in comparative water consumption. The statistical parameters highlight the significance of these data. The weight curve and food consumption did not change.

V-A si **V-E** extracts weren't toxic for the administered doses. It is observed from **Table 4** that LD50 value could be determined for **V-AE** extract on the studied dose sequence. According to Hodge-Stern toxicity scale, the LD50 value of **V-AE** extract is in the slightly toxic category (Ahmed M., 2015). **V-A** and **V-E** extracts were not found to be toxic on the administered dose sequences.

Table 1.

Assessment of body weight, eating behaviour and water consumption after administration of V-A extract

| Crt. no. | Administered drug | Parameter | Average / animal D1 | Average / group D14 | Average / animal D14 | Statistical parameters (water consumption) |
|----------------|--------------------------------|--------------------------------|---------------------|---------------------|----------------------|--|
| Group 1 | V-A extract 800 mg/kg b.w. | Weight b.m. (g) / group | 39.66 | 115.92 | 38.64 | P value 0.0040 Mean ± SEM of column A 3.003 ± 0.3317, n=3 Mean ± SEM of column B 12.23 ± 1.518, n=3 Difference between means 9.227 ± 1.554 95% confidence interval 4.913 ↔ 13.54 t=5.939, df=4 * SEM, Standard error of mean |
| | | Food consumption (g) / group | 4.00 | 17.85 | 5.95 | |
| | | Water consumption (mL) / group | 3.33 | 45.00 | 15.00 | |
| Group 2 | V-A extract 1600 mg/kg b.w. | Weight b.m. (g) / group | 39 | 92.71 | 37.88 | |
| | | Food consumption (g) / group | 6.34 | 14.28 | 5.71 | |
| | | Water consumption (mL) / group | 3.34 | 29.82 | 11.92 | |
| Group 3 | V-A extract 3200 mg/kg b.w. | Weight b.m. (g) / group | 34 | 105.42 | 35.14 | |
| | | Food consumption (g) / group | 6 | 16.07 | 5.35 | |
| | | Water consumption (mL) / group | 2.34 | 29.32 | 9.77 | |

Table 2.

Assessment of body weight, eating behaviour and water consumption
after administration of V-AE extract

| Crt. no. | Administered drug | Parameter | Average / animal D1 | Average / group D14 | Average / animal D14 | Statistical parameters (water consumption) |
|----------|---------------------------------|--------------------------------|---------------------|---------------------|----------------------|--|
| Group 1 | V-AE extract 800 mg/kg b.w. | Weight b.m. (g) / group | 37.00 | 109.05 | 37.40 | P value 0.1578 Mean \pm SEM of column A 7.223 \pm 2.001, n=3 Mean \pm SEM of column B 10.76 \pm 0.3824, n=3 Difference between means 3.533 \pm 2.037 95% confidence interval -2.122 \leftrightarrow 9.188 t=1.735 df=4 * SEM, Standard error of mean |
| | | Food consumption (g) / group | 6.34 | 17.14 | 5.85 | |
| | | Water consumption (mL) / group | 3.34 | 29.82 | 10.18 | |
| Group 2 | V-AE extract 1600 mg/kg b.w. | Weight b.m. (g) / group | 34 | 106.00 | 35.33 | |
| | | Food consumption (g) / group | 6.00 | 17.92 | 5.97 | |
| | | Water consumption (mL) / group | 10 | 34.46 | 11.48 | |
| Group 3 | V-AE extract 3200 mg/kg b.w. | Weight b.m. (g) / group | 32.33 | 47.82 | 22.11 | |
| | | Food consumption (g) / group | 5.00 | 6.57 | 4.18 | |
| | | Water consumption (mL) / group | 8.33 | 16.64 | 10.61 | |

Table 3.

Assessment of body weight, eating behaviour and water consumption
after administration of V-E extract

| Crt. no. | Administered drug | Parameter | Average / animal D1 | Average / group D14 | Average / animal D14 | Statistical parameters (water consumption) |
|----------|--------------------------------|--------------------------------|---------------------|---------------------|----------------------|---|
| Group 1 | V-E extract 800 mg/kg b.w. | Weight b.m. (g) / group | 37.33 | 112.25 | 37.41 | P value 0.0032 Mean \pm SEM of column A 2.78 \pm 0.56, n=3 Mean \pm SEM of column B 12.32 \pm 1.394, n=3 Difference between means 9.54 \pm 1.503 95% confidence interval 5.368 \leftrightarrow 13.71 t=6.349 df=4 * SEM, Standard error of mean |
| | | Food consumption (g) / group | 6.66 | 22.14 | 7.38 | |
| | | Water consumption (mL) / group | 3.34 | 44.42 | 14.82 | |
| Group 2 | V-E extract 1600 mg/kg b.w. | Weight b.m. (g) / group | 35.00 | 112.28 | 37.42 | |
| | | Food consumption (g) / group | 7.33 | 16.92 | 5.64 | |
| | | Water consumption (mL) / group | 1.66 | 30.00 | 10.00 | |
| Group 3 | V-E extract 3200 mg/kg b.w. | Weight b.m. (g) / group | 31.00 | 110.92 | 36.97 | |
| | | Food consumption (g) / group | 9.00 | 20.5 | 6.83 | |
| | | Water consumption (mL) / group | 3.34 | 36.42 | 12.14 | |

Table 4.

Determination of LD50 for V-A, V-AE, V-E extracts for acute toxicity assessment

| Crt. no. | Administered drug | Mortality | LD50 value (mg/kg b.w.) |
|----------|-------------------|-----------|---|
| 1 | V-A extract | 1/9 | n/a |
| 2 | V-AE extract | 2/9 | 2021.09 +/- 1484.2 Y = 0.246 + 1.438*X |
| 3 | V-E extract | 0/9 | n/a |

3. Conclusions

Having in view the potential anti-ulcer and analgesic action of the plant reported in literature, we initiated a preliminary study to assess acute toxicity and to determine LD50 values for the obtained extracts. Demonstration of the LD50 value for **V-AE** extract and additionally the assessment of water and food consumption and body weight allow further study for advanced research.

The significant change in water consumption for **V-A** and **V-E** extracts demonstrated in this experiment cannot be explained yet. The recorded data encourage further research on vegetal material in terms of phytochemical characterization and the assessment of some potential biological actions.

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