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Phytochemical Screening and Toxicological Study of *Neptunia oleracea* Lour. (Mimosaceae) Extracts, Plant Used in Traditional Medicine

Geoffroy G. Ouedraogo[1*], Gaétan D. Somda[2], Sylvain Ilboudo[1,3], Noufou Ouedraogo[1,3], Jean Koudou[4] and Pierre I. Guissou[5]

[1]Département de Médecine et Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé, Centre National de la Recherche Scientifique et Technologique (MEPHATRA-PH/IRSS/CNRST), 03 BP 7192 Ouagadougou 03, Burkina Faso.
[2]Laboratoire de Toxicologie, Environnement et Santé (LATES), Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
[3]Unité Mixte de Recherche Internationale - Environnement, Santé, Sociétés (UMI 3189, ESS) CNRST/CNRS/UCAD/UGB/USTTB, Burkina Faso.
[4]Université Aube Nouvelle, 06 BP 9283 Ouagadougou 06, Burkina Faso.
[5]Université Saint Thomas d'Aquin, 06 BP 10212 Ouagadougou 06, Burkina Faso.

Authors' contributions

This work was carried out in collaboration among all authors. Author GGO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GDS managed the literature searches and carried out the different experiments. Authors SI and NO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Neptunia oleracea (Lourd.) *Mimosaceae* is a plant commonly used in traditional medicine for the treatment of several pathologies such as dysentery, jaundice, leucorrhoea, troubles of earache, among others.
Aim: The purpose of this study was to carry out preliminary phytochemical screening, acute toxicity studies and to evaluate the effect of the aqueous and hydro-ethanolic extracts of *N. oleracea* on intestinal motility *in vivo*.

**Corresponding author: E-mail: guioffray@yahoo.fr;*

Methodology: Preliminary qualitative phytochemical screening was conducted using standard procedures while acute toxicity studies was performed using OECD method. The effect of *N. oleracea* extracts on intestinal motility was evaluated using on normal and acetylcholine-induced transits.

Results: Preliminary qualitative phytochemical screening of aqueous and hydro-ethanolic extracts of *N. oleracea* revealed the presence of similar constituents including steroids, triterpenoids, saponins, tannins, flavonoids, anthocyanidins, coumarins and carbohydrates. Alkaloids was absent in both the extracts. The oral median lethal dose (LD_{50}) for both extracts was estimated to be 5000 mg/kg.

The effect of extracts on intestinal peristalsis in mice showed that the aqueous and hydro-ethanolic extracts of *N. oleracea* stimulate normal intestinal transit by 1.29 and 8.54% respectively at the dose of 50 mg/kg body weight, thus there was inhibition at higher doses. These extracts potentiate acetylcholine-induced intestinal transit by 23.9 and 14.39% respectively at 500 mg/kg body weight.

Conclusion: The findings of this study showed that the aqueous and hydro-ethanolic extracts of *Neptunia oleracea* contain bioactive constituents that have practically no toxic effect. This could justify the many forms of use of this plant in traditional medicine.

Keywords: Neptunia oleracea; acute toxicity; NMRI mice; intestinal transit; Burkina Faso.

1. INTRODUCTION

The therapeutic use of medicinal plants is still very present in some countries of the world and especially in developing countries despite the progress of pharmacology [1]. In Africa, medicinal plants are valuable resources for the majority of rural populations, where more than 80% use these plants for health care [2]. In Burkina Faso, traditional medicine and pharmacopoeia are the main source of primary health care for 70% of the population [3]. *Neptunia oleracea* (*N. oleracea*) is one of the many plants used in traditional medicine in Burkina Faso to treat different ailments including dysentery, skin diseases, syphilis, earaches and guinea worm infections [4].

Several authors have expressed interest in the pharmacological and chemical aspect of this plant. Thus, phytochemical studies have revealed several chemical groups including flavonoids, anthraquinones and tannins in the aqueous and hydro-ethanolic extracts from *N. oleracea* leaves [5,6]. Pharmacological studies have shown that *N. oleracea* has astringent, antimicrobial, anti-tumor and hepatoprotective properties [5,7,8].

However, there are very few data on the toxicity of this plant. Several studies around the world have however reported serious side effects related to the use of medicinal plants [9]. Toxic drugs cause serious liver damage and are responsible for about 10% of acute liver failure and 5% of itching [10,11]. In Morocco, plant intoxications are responsible for 14.2% of deaths [12]. In Burkina Faso, more than 22% of renal failure is due to medicinal plants [13]. However, some traditional health practitioners and populations are not always aware of the toxicity of medicinal plants. Safety and security are therefore important criteria to consider before administering herbal products. That is why WHO recommends that medicinal plants should be studied to better understand their therapeutic properties and to ensure their safe use [14]. The purpose of this work was therefore to carry out the phytochemical profile and evaluate the toxicity of *N. oleracea* extracts to allow a safety use of this medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material of *N. oleracea* leaves was harvested in August 2014 in Ouagadougou (Burkina Faso). It was identified and authenticated at the Burkina Faso National Herbarium (HNBU) where a voucher specimen was deposited under N°. 8729. The leaves were shade-dried and pulverized using a laboratory crusher (Blade Crusher, Gladiator East. 1931 Type BN 1 Mach. 40461 1083). The vegetable powder obtained was used to prepare aqueous and hydro-ethanolic extracts.

2.2 Experimental Animals

Toxicological studies were conducted on male and female NMRI mice weighing between 23 and 31 g. The animals were obtained from the ''Institut de Recherche en Sciences de la Santé''

(IRSS) pet shop and were reared in controlled room temperature (23-25°C) with 40-65% of humidity. They were fed with protein enriched wheat cake (29%) and tap water. These animals were evenly distributed per sex in cages containing three mice each and they were subjected to 12 hours of illumination and 12 hours of darkness.

2.3 Aqueous Decoction Preparation

A portion (100 g) of the vegetable powder was introduced into a flask containing 700 mL of distilled water. The mixture was heated under reflux for 1 hour. At the end of this operation, the decocted extract obtained was filtered using a nylon fabric and then centrifuged at 2000 rpm for 10 minutes. The filtrate obtained was dried in an oven at 45°C under ventilation. The dried extract obtained was weighed to determine the extraction yield.

2.4 Hydro-ethanolic Decoction Preparation

A sample (100 g) of vegetable powder was placed in a flask containing 500 mL of 80% hydro-ethanolic solution. The mixture was boiled under reflux for 1 hour. The decoction, after cooling, was filtered on Whatman N°5 paper and concentrated with rotary evaporator at 60 to 70°C. The concentrated extract was dried in an oven at 45°C under ventilation and weighed to determine the extraction yield.

2.5 Phytochemical Screening

The aqueous and hydro-ethanolic extracts of *N. oleracea* were subjected to preliminary phytochemical screening according to the method described by Ciulei [15] and adapted by the Phytochemistry Laboratory of the Institute for Research in Health Sciences (IRSS).

2.6 Acute Toxicity Studies

The acute toxicity study was conducted using the OECD N° 423 guideline [16]. The test was performed on two groups of three healthy female mice weighing between 23 and 31 g. The mice were fasted four hours prior the test.

A single dose of 2000 mg/kg body weight (b.w.) of each extract was administered orally using a gastric tube. After administration of the extracts, the animals were observed every 30 minutes for 2 hours. After the two hours observation, the animals were fed and then observed daily for 14 days. Mortality and any behavioral change such as changes in skin and fur, eyes, mucus membranes, convulsion, salivation, diarrhoea, lethargy, sleep and coma were recorded during the observation period.

2.7 Effects of *N. oleracea* Extracts on Intestinal Peristalsis *in vivo*

The study of the effects of *N. oleracea* aqueous and hydro-ethanolic extracts on intestinal motility evaluated the effect of these extracts on normal and acetylcholine-induced transit. This study was carried out according to an adaptation of the protocol described by Tagne et al. [17].

The normal transit study was performed using four groups of four mice each. The animals were fasted for 18 hours before administration of the extracts. The first group (negative control) received 0.5 mL of 40% activated charcoal in distilled water, orally. The three other groups were administered with either loperamide at 5 mg/kg, extract at dose of 50 mg/kg and/or 500 mg/kg b.w., orally. The mice from these three groups received 0.5 mL of 40% activated charcoal 30 minutes after extracts administration. All the mice were sacrificed 30 minutes after the charcoal administration. The distance travelled by the charcoal in the small intestine and the whole length of the intestine were measured.

Intestinal transit was calculated according to the following formula:

$$\% \text{ Intestinal transit} = \frac{\text{distance travelled by activated charcoal}}{\text{total lenght of the small intestine}} * 100$$

For acetylcholine-induced intestinal transit study, three (3) groups of four mice each per extract were used. After 18 hours fasting, 0.1 mg/kg of acetylcholine was administered intraperitoneally (*i.p.*) to the first group (positive control). The two other groups received acetylcholine at 0.1 mg/kg *i.p*. followed by the extract at 50 and 500 mg/kg b.w. respectively *per os*.

Thirty (30) minutes later, all the mice received 0.5 mL of activated charcoal at 40% in distilled water. The mice were sacrificed 30 minutes after the activated charcoal administration. The distance travelled by the activated charcoal in the small intestine and the total length of the small intestine were measured.

Intestinal transit was calculated according to the following formula:

% Intestinal transit =

$$\frac{\text{distance travelled by activated charcoal}}{\text{total lenght of the small intestine}} * 100$$

2.8 Statistical Analysis

Both qualitative and quantitative data were presented in tables. The results of *in vivo* test were expressed as mean ± SD (Standard deviation). The statistical analyses of variance were done by ONE WAY ANOVA followed by the Dunnett's multiple comparison tests through the Graph Pad Prism 5.0 program. Differences were considered significant if $p < 0.05$.

3. RESULTS

3.1 Percentage Yields

After the aqueous and hydro-ethanolic decoctions preparation the extraction yields were determined. The extraction yields in terms of dry extract ranged from 16.06% to 0.54% (Table 1).

3.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening of *N. oleracea* conducted on the aqueous and hydro-ethanolic extracts revealed the presence of similar chemical constituents such as flavonoids, saponins, tannins, coumarins, and carbohydrates (Table 2).

3.3 Oral Acute Toxicity Study of Aqueous and Hydro-ethanolic Extracts of *N. oleracea*

The acute toxicity study showed that *N. oleracea* extracts did not cause mortality in NMRI mice when administered orally with 2000 mg/kg b.w. No symptoms of intoxication related to the extracts was observed during the 72 hours of observation following the administration of the extracts and after two weeks of observation (Table 3).

In view of the results of Table 3, the LD_{50} of *N. oleracea* extracts was estimated to be 5000 mg/kg b.w. when administered orally, according to the OECD test guidelines.

3.4 Effects of Aqueous and Hydro-ethanolic Extracts from *N. oleracea* on Intestinal Transit *in vivo*

The effect of aqueous and hydro-ethanolic extracts from *N. oleracea,* on normal intestinal transit is presented in Table 5. The results showed that the extracts (aqueous and hydro-ethanolic) at 50 mg/kg b.w. stimulated the normal

Table 1. Percentage yield of *N. oleracea* aqueous and hydro-ethanolic extract

Extracts	Hydro- alcoholic decoction (%)	Aqueous decoction (%)
Crude extraction	15.93 ± 0 .35	16.06 ± 1.59

Table 2. Preliminary phytochemical screening of *N. oleracea* extracts

Chemical compounds	Aqueous decoction	Hydro-ethanolic decoction
Steroids and triterpenoids	+	+
Saponins	+	+
Polyphenolic compounds (tannins)	++	++
Flavonoids	±	+
Anthocyanidins	+	+
Coumarins	+	+
Alkaloids	-	-
Carbohydrates	+	+

Key: ++ = abundant; + = scarce; ± = trace; - = absent

Table 3. Oral acute toxicity study of *N. oleracea* aqueous and hydro-ethanolic extracts

Extract	First test		Second test	
	Mortality	Mortality rate (%)	Mortality	Mortality rate (%)
Aqueous decoction	0/3	0	0/3	0
Hydro-ethanolic decoction	0/3	0	0/3	0

transit of 1.29 and 8.54% respectively while acetylcholine stimulated the transit of 9.68% at dose of 0.1 mg/kg b.w. However, at 500 mg/kg b.w., these extracts inhibited normal transit of 2.59 and 2.07% for aqueous and hydro-ethanolic extracts, respectively. Loperamide inhibited this transit of 20.18% at 5 mg/kg b.w.; the normal intestinal transit was 64.17 ± 8.05 [15].

Table 5 presents the results of acetylcholine-induced intestinal transit test. The results indicated that the aqueous and hydro-ethanolic extracts from *N. oleracea* potentiated the intestinal transit induced by acetylcholine. At a dose of 50 mg/kg, aqueous and hydro-ethanolic extracts from *N. oleracea* increased acetylcholine-induced intestinal transit of 10.85 and 7.04% respectively. At 500 mg/kg body weight, the aqueous extract increased acetylcholine-induced intestinal transit of 23.90%. At the same dose, hydro-ethanolic extract stimulated acetylcholine-induced intestinal transit of 14.39%.

4. DISCUSSION

Plants produce a variety of natural substances including secondary metabolites to protect against predators and pathogens. These secondary metabolites are most often responsible for the toxicity of certain plants [18]. According to some authors, 20% of plants were once used as abortive, 20% for criminal purposes, 15% for witchcraft and 10% for psychoactive plants [19]. Among the toxic secondary metabolites of plants are coumarins, which have spasmolytic properties. Some of them (hydroxycoumarins) cause haemorrhagic diarrhea, haematuria and dyspnea, which can lead to death. Toxic diterpenoids induce violent digestive disorders or severe skin or eye irritations. Tannins cause reduced growth in animals by inhibiting the metabolic use of amino acids after absorption [20].

Phytochemical screening of aqueous and hydro-ethanolic extracts of *N. oleracea* leaves revealed the presence of several chemical constituents including steroids, flavonoids, tannins, saponins, etc. These results are in consistent to what was reported by other authors for the different parts of this plant [5,21,22].

In the oral acute toxicity study, the estimated LD50 was 5000 mg/kg for the aqueous and hydro-ethanolic extracts from N. oleracea. These extracts can be classified in category 5, ie substances unlikely to present acute hazard according to the Globally Harmonized System of Classification and Labeling of Chemicals of the United Nations [23].

The results is in agreement with those of previous studies which has noted the absence of mortality up to dose of 2000 mg/kg aqueous and hydro-ethanolic extracts of *N. oleraceae* in single oral administration [5,7]. This low toxicity could support the multiple uses of this plant in traditional medicine.

Table 4. Effect of *N. oleracea* extracts on normal intestinal transit

Treatment	Dose (mg/kg)	Intestinal transit	Inhibition rate (%)	Stimulation rate (%)
Normal control		64.17 ± 8.05		
Acetylcholine	0.1	73.84 ± 4.63		9.68
Loperamide	5	43.98 ± 4.81	20.18	
Aqueous extract	50	65.45 ± 12.98		1.29
	500	61.57 ± 8.79	2.59	
Hydro-ethanolic extract	50	72.71 ± 3.10		8.54
	500	62.09 ± 1.77	2.07	

Table 5. Effect of aqueous and hydro-ethanolic extracts from *N. oleracea* on acetylcholine-induced intestinal transit

Treatment	Dose (mg/kg)	Intestinal transit	Stimulation rate (%)
Normal control		64.17 ± 8.05	
Acetylcholine	0.1	73.84 ± 4.63	9.68
Aqueous extract	50	75.02 ± 4.09	10.85
	500	88.07 ± 7.65	23.90
Hydro-ethanolic extract	50	71.21 ± 3.48	7.034
	500	78.56 ± 3.45	14.39

However, since this study focused on acute toxicity, repeated-dose toxicity studies will provide a better understanding of the toxic potential of these extracts.

The results of the intestinal transit study showed that acetylcholine stimulated intestinal transit. The aqueous and hydro-ethanolic extracts of *N. oleracea* stimulated normal intestinal transit at low dose while at high doses both extracts inhibited normal transit. Also these extracts stimulated acetylcholine-induced intestinal transit.

The gastrointestinal peristalsis is controlled by the cholinergic system, of which acetylcholine is one of the neurotransmitters [24]. It is synthetized by enteric excitatory motor neurons and its binding to the M3 receptor of the gastrointestinal tract leads to an increase of motility, tone and intestinal peristalsis [25]. Acetylcholine has ability to activate parietal cells and G cells as well as enterochromaffin cells (ECL). G cells and ECL cells produce gastrin and histamine respectively. Gastrin, histamine and acetylcholine are hormones that promote digestion by stimulating the secretion of protons. In addition, acetylcholine and cholinergic agonists by activating muscarinic M3 and M1 receptors inhibit the absorption of sodium and chloride ions and stimulate the secretion of these ions and water into the colon [26].

Apart from acetylcholine, vasoactive intestinal peptide (VIP), nitric oxide (NO) and ATP are neurotransmitters synthesized by inhibitory neurons whose release induces muscle relaxation of the gastrointestinal tract [27]. The aqueous and hydroethanolic extracts of *N. oleracea* is believed to act as an acetylcholine agonist on M3 muscarinic receptors, G cells or ECL cells in the gastrointestinal tract, increasing the tone and contractions of the intestine, resulting in increased intestinal transit. This mechanism may also explain the exacerbation of the effect of acetylcholine on intestinal transit. The inhibition of normal intestinal transit by aqueous and hydro-ethanolic extracts from *N. oleracea* at high-dose would be related to the capacity of metabolites present in these extracts to occupy other receptors whose activation would cause adverse effects of acetylcholine such as opioid receptors (μ). Indeed, the activation of muscular opioid receptors (μ) in the gastrointestinal tract reduces motility and propulsive contractions and gastric emptying, but leads to an increase in muscle tone and non-propulsive (segmental) contractions [28]. It is also possible that these extracts, in high doses, inhibit intestinal motility by acting directly on circular muscles and long intestinal muscles such as loperamide [29]. The traditional use of *N. oleracea* could cause diarrhea to the patient due to the ability to exacerbate the effect of acetylcholine, or constipation if given at high doses.

According to Mamyrbekova – bekro et al. [30], coumarins, anthraquinones and alkaloids are believed to have purgative properties. However, phytochemical analysis revealed the presence of coumarins and phenolic compounds in aqueous and hydroethanolic extracts from *N. oleracea*. The presence of these compounds could justify the effect of *N. oleracea* extracts on gastrointestinal transit.

5. CONCLUSION

This study indicated the presence of different chemical constituents in aqueous and hydro-ethanolic decoctions from *N. oleracea* leaves; and the acute oral toxicity study of the aqueous and hydro-ethanolic extracts from *N. oleracea* was found to be safe orally. The extracts stimulated basic bowel contractions at low dose but induced inhibition of these contractions at high dose. In view of these results, it can be concluded that the many forms of use of this plant in traditional medicine can be justified by its richness in secondary metabolites and its low toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocol was carried out in accordance with international standard protocols [Guidelines set by the European Union on the protection of animals (CEC Council 86/609)] and adopted by IRSS, Burkina Faso. These different experiments were carried out on the mice and did not concern in any case the human subject. These protocols are ethical to experiment on laboratory animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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