Original Research Article

Phytochemical and toxicological study of *Neptunia oleracea* Lour. (Mimosaceae) extracts, plant use in traditional medicine

.

ABSTRACT

Neptunia oleracea (Lourd.) *Mimosaceae* is a plant commonly used in traditional medicine. This plant was fluently used in traditional medicine for the treatment of several pathologies such as dysentery, jaundice, leucorrhoea, troubles of eareache, etc.

Aims: the purpose study was to carried out the phytochemical profile, to study the acute toxicity of extracts from this medicinal plant and evaluate the effect of these extracts on intestinal motility *in vivo*

Methodology: Phytochemical screening and acute toxicity test were performed using standards methods. The effects of *N. oleracea* extracts on intestinal motility consisted in evaluating the effect of these extracts on normal and acetylcholin-induced transit.

Results: Phytochemical screening revealed the presence of several chemical groups including steroids, triterpenes, flavonids, tannins, saponins, etc.

The toxicological study gave an estimated LD50 of 5000 mg/kg for all extracts. The investigation of effect of extracts on intestinal peristalsis in mice showed that the aqueous and hydro-ethanolic extracts of *Neptunia oleracea* stimulate normal intestinal transit by 1.29 and 8.54% respectively at the dose of 50 mg/kg b.w. but inhibit it at higher doses. These extracts potentiate acetylcholin-induced intestinal transit by 23.9% and 14.39% respectively at 500 mg/kg body weight.

Conclusion: this study showed that the extracts of *Neptunia oleracea* contain secondary metabolites and 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% uses theses 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. This plant is used to treat many diseases 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 flavonids, 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 and 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. It is recognized that toxic drugs cause serious liver damage and are responsible for about 10% of acute liver failure and 5% of itching [9, 10]. In Morocco, plant intoxications are responsible for 14.2% of deaths [11]. According to the Pharmacotherapeutic Information Bulletin of Burkina Faso (2015), more than 22% of renal failure is due to medicinal plants. 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 [12]. The purpose of this work was therefore to evaluate the toxicity of *N. oleracea* extracts to allow a better safety of it use in traditional medicine.

2. MATERIAL AND METHODS

2.1. Plant Material

The plant material consisted of *N. oleracea* leaves harvested in august 2014 in Ouagadougou (Burkina Faso). The plant was identified and authentified at the Burkina Faso National Herbarium (HNBU) where a voucher specimen was deposited under N°. 8729. The leaves were dried in shade, away from dust and then crushed 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. Animal

Toxicological studies was 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 subjected to 12 hours of illumination and 12 hours of darkness. The mice were evenly distributed per sex in cages with three mice per cage.

2.3. Aqueous decoction preparation

A portion of 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 dry extract obtained was weighed to determine the extraction yield.

2.4. Hydro-ethanolic decoction preparation

A sample of 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 Wattman N°5 paper and concentrated with rotavapor 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. Alkaloids-rich fraction preparation

The alkaloids-rich fraction was prepared according to the protocol described by Mbodj (2003) [13]. To 100 mL of the aqueous or hydro-ethanolic decoction was added a sodium carbonate solution (Na_2CO_3) at 5% until pH8. The mixture was transferred into 250 mL separating funnel and extracted by liquid/liquid sharing with 3x 25 mL of chloroform. After settling, the organic phase was recovered and filtered on filter paper. The filtrate obtained was concentrated under reduced pressure with rotavapor. The dry extract obtained constitutes the alkaloidal fraction.

2.6. Phytochemical screening

The phytochemical screening was performed on the extracts in solution using the method described by Ciulei (1982) [14] and adapted by the phytochemistry laboratory of the Institute for Research in Health Sciences (IRSS).

2.7. Acute toxicity study

The acute toxicity study was conducted using the OECD N° 423 guideline [15].
The test was performed on two groups of three healthy female mice weighing between 23 and 31 g. The mice were fasted four hours before 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 behavourial change were recorded during the observation period.

2.8. 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 consisted in evaluating the effect of these extracts on normal and acetylcholin-induced transit. This study was carried out according to an adaptation of the protocol described by Tagne *et al.* (2015) [16] .
The normal transit study was performed using four groups of mice. The animals were fasted for 18 hours after which we administered the extracts. The first group (negative control) received orally 0.5 mL of 40% activated charcoal in distilled water. The three other groups were administered respectively loperamide at 5 mg/kg, extract at dose of 50 mg/kg and 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 acetylcholin-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 were administered intraperitoneally (i.p.) to the first group (positive control). The two other groups received acetylcholine at 0.1 mg/kg *i.p*. and the extract respectively at 50 and 500 mg/kg b.w.
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:

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

2.9. 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. Extraction 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).

Table 1: Extraction yields from *N. oleracea* aqueous and hydro-ethanolic decoction yields (n=3)

Extracts	Hydro-alcoolic decoction (%)	Aqueous decoction (%)
Crude extraction	15.93 ± 0 .35	16.06 ± 1.59
Alkaloids fraction	0.54	0.56

3.2. Phytochemical screening

The results of phytochemical screening of *N. oleracea* aqueous and hydro-ethanolic extracts showed the presence of several chemical groups such as flavonoids, saponins tannins, coumarins, and carbohydrates (Table 2).

Tableau 2: phytochemical profil of *N. oleracea* aqueous and hydro-ethanolic decoction

Chemical compounds	Aqueous decoction	Hydro-ethanolic decoction
Steroids and triterpinoids	+	+
Saponins	+	+
Polyphenolic compounds (tannins)	++	++
Flavonids	±	+
Anthocyanosides	+	+
Coumarins	+	+
Alcaloids	-	-
Carbohydrates (oses)	+	+

++ = abundant; **+** = scarce; **±** = trace; - = absent

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

The acute toxicity study showed that *N. oleracea* extracts did not caused mortality in NMRI mice when administered orally at 2000 mg/kg b.w. Any symptoms of intoxication related to the extract were not observed during the 72 hours of observation following the administration of the extracts and after two weeks of observation. The results of the oral acute toxicity of *N. oleracea* extracts are presented in table 3.

Table 3: Results of oral acute toxicity study of *N. oleracea* aqueous and hydro-éthanolic extracts in mice at 2000 mg/kg b.w. (n=3)

Type of extracts	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

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

3.4. Oral acute toxicity of the alkaloids fraction of N. oleracea aqueous and hydro-ethanolic extracts

The oral administration of the alkaloids fraction of *N. oleracea* at 2000 mg/kg b.w. did not caused mortality or symptoms of intoxication (Table 4).

Table 4: oral acute toxicity of the alkaloids fractions from *N. oleracea* aqueous and hydro-ethanolic extracts in mice at 2000 mg/kg b.w. (n=3).

First test		Second test	
Mortality	Mortality rate (%)	Mortality	Mortality rate (%)
0/3	0	0/3	0

The oral LD_{50} of the alkaloids fraction of aqueous and hydro-ethanolic extracts of *N. oleracea* is estimated to be 5000 mg/kg b.w. according to the OECD (2001) test scheme.

3.5. Effects of aqueous and hydro-ethanolic extracts from *N. oleracea* on intestinal transit *in vivo*

The results of the effects of aqueous and hydro-ethanolic extracts from *N. oleracea,* on normal intestinal transit, were presented in table 5. These results show that at 50 mg/kg b.w, aqueous and hydro-ethanolic extracts from *N. oleracea* stimulated the normal transit of 1.29 and 8.54% respectively while acetylcholine stimulated this transit of 9.68% for at dose of 0.1mg/kg b.w. However, at 500 mg/kg b.w., these extracts inhibited normal transit of 2.58 and 2.07% respectively for aqueous and hydro-ethanolic extracts. Loperamide inhibited this transit of 20.18% at 5 mg/kg b.w.; the normal intestinal transit was 64.17 ± 8.05.

Table 5: 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 6 presents the results of acetylcholin-induced intestinal transit test. These results show 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 acetylcholin-induced intestinal transit of 10.85 and 7.04% respectively. At 500 mg/kg body weight, the aqueous extract increased acetylcholin-induced intestinal transit of 23.90%. At the same dose, hydro-ethanolic extract stimulated acetylcholin-induced intestinal transit of 14.39%.

Table 6: Effect of aqueous and hydro-ethanolic extracts from *N. oleracea* on acetylcholin-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

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 of the toxicity of certain plants [17]. According to some authors, 20% of plants were once used as abortive, 20% for criminal purposes, 15% for witchcraft and 10% for psychoactive plants [18]. Among the toxic secondary metabolites of plants are coumarins, which have spasmolytic properties. Some of them (hydroxicoumarins) 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 [19].

Phytochemical screening of aqueous and hydro-ethanolic extracts of *N. oleracea* leaves revealed the presence of several chemical groups including steroids, flavonids, tannins, saponins, etc. These results support those of other authors who have found the same chemical groups in different extracts from different parts of this plant [5, 20, 21].

The results of the oral acute toxicity of aqueous and hydro-ethanolic extracts from *N. oleracea* and the alkaloids fractions showed that at 2000 mg/kg b.w., these extracts did not cause mortality in mice. During the observation period, no signs of intoxication were observed. The oral LD_{50} of these extracts is estimated to be 5000 mg/kg. These extracts can be classified in category 5 of substances not known to present an acute hazard according to the WHO classification [22]; the globally harmonized OECD and United Nation classification system [15, 23].

These results support those of other authors who have shown that aqueous and hydro-ethanolic extracts of *N. oleracea* do not cause mortality in rats at 2000 mg/kg b.w. when administrate orally [5, 7]. The results of our work confirm that the aqueous and hydro-ethanolic extracts of *N. oleracea* and their alkaloids fractions are weakly toxic as found in previous works. This low toxicity could support the multiple uses of this plant in traditional medicine.

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 of 9.68% at 0.1 mg/kg. 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. At 50 and 500 mg/kg, these extracts stimulated acetylcholin-induced intestinal transit.

The motricity of the gastrointestinal tract 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 (mus (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 [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 allowed us to identify the different chemical groups present in aqueous and hydro-ethanolic decoctions from *N. oleracea* leaves, to know the oral LD_{50} of crude extracts and the alkaloids fractions and to evaluate the interaction of these extracts on the smooth muscle motricity. The phytochemical study revealed the presence of various chemical compounds such as steroids, triterpenes, anthocyanosides, coumarins, tannins, saponins, flavonids, etc. The acute oral toxicity study showed that this plant is weakly toxic by oral route. The evaluation of the effect of the smooth muscle motricity showed that aqueous and hydro-ethanolic extracts from *N. oleracea* stimulate basic bowel contractions at low dose but induce inhibition of these contractions at high dose. In view of these results, we can say 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

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.

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