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Phytochemical Screening, Acute Toxicity and Anti-Diarrhoeal Effect of Extracts from *Prosopis africana* (Guill. & Perr.) Taub (Mimosaceae)

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Abstract: ***Prosopis africana is one of the plants commonly used in traditional medicine in Burkina Faso. Several studies have shown that this plant contains many compounds, which gives it different pharmacological properties. The objective of this work was to determine the phytochemical and toxicological profile and also the effect of extracts from this medicinal plant on intestinal peristalsis in vivo in mice using standards methods. Phytochemical screening has revealed the presence steroids, triterpenes, anthocyanosides, tannins, saponosides, reducing compounds, carbohydrates, etc. The acute toxicity studies were permit to estimated LD_{50} of 5000 mg/kg b.w. for the aqueous and hydroethanolic crude extracts and 1000 mg/kg for alkaloid-enriched fraction of aqueous extract. The study of intestinal peristalsis showed that aqueous and hydroethanolic extracts of Prosopis africana inhibited intestinal transit by 15.36% and 25.84%, respectively, at a dose of 500 mg / kg. At the same dose, the hydro-ethanolic extract potentiated acetylcholine-induced intestinal transit by 21.56% while the aqueous extract inhibits it. This study showed that Prosopis africana extracts are rich in secondary metabolites and are virtually harmless. This could justify this plant many uses in traditional medicine.***

Keywords: *Prosopis africana*, Acute toxicity, Wistar rat, Intestinal transit, Burkina Faso

1. Introduction

Prosopis africacna (Guill. & Perr.) Taub (*P. africana*), belonging to Mimosaceae family is a plant commonly used in traditional medicine in Burkina Faso. It is used to treat dysentery, stomach pain, headaches, toothache, rheumatism, skin diseases, cavities, fever, gonorrhea, bronchitis, etc. [1], [2].

Several studies concerning the pharmacological and chemical aspects have already been carried out on this plant.

Phytochemical studies have shown the presence of saponosides, flavonoids, tannins, carbohydrates, alkaloids, phenols and steroids in various extracts of *P. africana* [3]–[6].

Pharmacological studies have shown that *P. africana* extracts have several interesting pharmacological activities including antimicrobial [3], anti-trypanosomal, and antiplasmodial properties [4], [7]. However, there is very few data on this plant toxicity. Altought, several studies carried out on traditional herbal treatments have reported a problem of toxicity or interaction that may cause therapeutic failures or accidents [8]. Different plants can be known as both toxic and medicinal plants [9]. A retrospective study conducted by the poison control center of Morocco showed a mortality rate of 14.2% for plant poisoning [10]. In Mali, 2.4% of acute intoxications in children during the period 2000-2010 were due to medicinal plants [11].

According to the pharmaco-therapeutic information bulletin of Burkina Faso (2015), more than 22% of renal patients used natural products. Thus, this purpose work was to evaluate the toxicity of extracts from this plant to allow a better safety use in traditional medicine.

2. Material and Methods

2.1. Plant material

The plant material consisted of *P. africana* leaves harvested in September 2014 in Ouéssa (IOBA province, Burkina Faso). The plant has been identified at the Burkina Faso National Herbarium (HNBU) where a specimen has been deposited under the voucher number No. 8728.

The leaves were dried in shade, dust-free and then pulverized using a laboratory mill (Blade Mill, East Gladiator, 1931 Type BN 1 Mach 40461 1083). The vegetable powder obtained was used for preparation of different extracts.

2.2. Animal

NMRI male and female mice weighing 25 to 34 g were used for this study. These animals were procured from the IRSS MEPHATRA-PH pet shop and were reared at an ambient temperature (23-25°C) and 40-60% of humidity. They were fed with 29% protein enriched wheat cake and tap water. These animals were subjected to 12 hours of illumination and 12 hours of darkness. They were homogeneously distributed

in polypropylene cages at a rate of three mice per cage and per sex.

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)].

2.3. Aqueous decoction preparation

An aqueous decoction was prepared by refluxing 100 g of the plant powder in 700 mL of distilled water. At the end of this operation, the decoction was filtered using a nylon fabric and then centrifuged at 2000 rpm for 10 minutes. The filtrate obtained is dried in an oven at 45°C under ventilation. The dry extract obtained was weighed to determine extraction yield.

2.4. Hydroethanolic decoction preparation

A portion of 100 g of plant powder was placed in a flask containing 500 ml of 80% hydroethanolic solution. The mixture was boiled under reflux for 1 h. The decoction obtained, after cooling, was filtered on Wattman N°5 paper and concentrated in a rotavapor at 60 to 70°C. The concentrated extract was dried in a ventilated oven at 45°C and weighed to determine extraction yield.

2.5. Alkaloids-rich fraction preparation

The alkaloids-rich fraction was prepared according to Mbodj, 2003.To 100 mL of aqueous or aqueous-alcoholic decoction was added a sodium carbonate solution (Na_2CO_3) at 5% until pH 8. The mixture was transferred in a 250 mL separatory funnel and extracted by liquid/liquid partition with 3 x 25 mL of chloroform. After decantation, the organic phase is recovered and filtered on filter paper. The filtrate obtained was concentrated under reduced pressure in a rotary evaporator. The dry extract obtained constitutes the alkaloid fraction.

2.6. Phytochemical screening.

The phytochemical screening was carried out according to the standard protocol as described by [12] and adapted at the Laboratory of Phytochemistry from the Institute of Research in Health Science (IRSS).

2.7. Acute toxicity test

Acute toxicity test was conducted using OECD N°423 guideline, the acute toxicity class method for the testing of chemicals [13].

This test was carried out on two groups of three healthy mice (both sexes) weighing between 25 and 34 g. The mice were fasted four hours before testing and then, a single dose of 2000 mg/kg of each extract was administered orally using a gastric tube. After extracts administration, the animals were observed every 30 minutes over the 2 hours following extracts administration. After two-hour observation the animals were fed and then observed daily for 14 days.

2.8. Effects of *P. africana* extracts on gastrointestinal motility *in vivo*

The effects of *P. africana* extracts on gastrointestinal motility were investigated in vivo by observing the interactions of extracts with normal intestinal transit and that induced by acetylcholine. In both approaches, intestinal transit study was carried out using the charcoal transit method according to an adaptation of the protocol described by[14].

For normal transit study, 4 groups of four mice were constituted. These animals were fasted 18 h before testing. After fasting period, the first group (negative control group) received 0.5 mL of 40% activated charcoal in distilled water. The mice were then sacrificed 30 min after the charcoal administration.

The second group received 5 mg/kg of loperamide (positive control). The two other groups received respectively, 50 mg/kg and 500 mg/kg body weight (b.w.) of plant extracts. Thirty (30) minutes later, 0.5 mL of 40% activated charcoal were administrated to mice. The animals were then sacrificed 30 min following charcoal administration. The distance traveled 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:

$$\%\ \text{Intestinal transit} = \frac{\text{distance traveled by activated charcoal}}{\text{total length of small bowel}} x\ 100$$

For acetylcholine-induced intestinal transit study, 3 groups of four mice each were used per extract. The mice were fasted 18 h before testing. The positive control group received intraperitoneal (*i.p.*) administration of acetylcholine (Ach) at dose of 1mg/kg b.w. The two other groups received 0.1 mg/kg of acetylcholine and respectively 50 and 500 mg/kg b.w. of plant extracts.

Thirty (30) minutes later, 0, 5 mL of a 40% charcoal were administrated.

The animals were then sacrificed thirty minutes after administration of charcoal. The distance traveled by the charcoal in the small intestine and the total length of the intestine were measured.

Intestinal transit was calculated according to the following formula:

$$\%\ \text{Intestinal transit} = \frac{\text{distance traveled by activated charcoal}}{\text{total length of small bowel}} x\ 100$$

2.9. Statistical analysis

Both qualitative and quantitative data was 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

Before phytochemical screening, the extraction yields of different extracts were determined. Table 1 shows the extraction yields in terms of solids obtain from crude extraction and alkaloid-enriched fraction.

Table 1: Aqueous and hydro-alcoholic decocts extraction yields

Extracts	*Hydro-alcoholic decoction*	*Aqueous decoction*
Crude extraction	24.93 ± 0.94	17.43 ± 0.35
Alkaloids fraction	0.58	3.08

3.2. Phytochemical Screening

The results of plant extracts phytochemical screening showed the presence of several chemical groups including steroidal, alkaloids, triterpenic, Saponosides, tannins coumarins, etc. in *P. africana* leaves aqueous and hydroethanolic extracts (Table 2).

Table 2: Phytochemical profile of. *P. africana* aqueous and hydroethanolic decoctions

Chemical compounds	*aqueous decoction*	*Hydroethanolic decoction*
Steroid and triterpernoids	++	++
Saponins	++	±
Polyphenolic compounds (Tannins)	+	+
Flavonids	-	±
Anthocyanosides	++	++
coumarins	++	+
Alkaloids	++	++

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

3.3. Oral acute toxicity study of aqueous and hydroethanolic decoction

Oral administration of *P. africana* total extracts caused not death at the single dose of 2000 mg/kg/bw of the NMRI mice during the 72 hours of observation following extracts administration and after two weeks of observation (Table 3). Also, no toxic symptoms in terms of behavioral changes, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss were observed in any animals, up to 14 days after the administration of plant extracts.

Table 3: Oral acute toxicity (LD_{50}) of *P. africana* in mice at 2000 mg/kg (n = 3)

Type of extracts	*first test*		*Second test*	
	mortality	*mortality rate (%)*	*mortality*	*mortality rate (%)*
Aqueous decoction	0/3	0	0/3	0
Hydroethanolic decoction	0/3	0	0/3	0

The oral LD_{50} of the *P. africana* total extract was estimated to be 5000 mg/kg body weight according to the OECD Test Guideline [13].

3.4. Oral acute toxicity study of the alkaloid-enriched fraction extract

Oral administration of the alkaloid-enriched fraction from *P. africana* leaves hydroethanolic extract at dose of 2000 mg/kg bw doesn't caused no death or sign of toxicity in the treated mice (Table 4).

However, the alkaloid-enriched fraction from *P. africana* leaves aqueous decoction was caused the death of two female mice 48h following administration at dose of 2000 mg/kg. At dose of 300 mg/kg b.w., this fraction doesn't cause any toxicity symptoms or death (Table 5).

Table 4: oral acute toxicity of the alkaloid-enriched fraction *from P. africana* hydroethanolic extract at 2000 mg/kg

First test		*Second test*	
Mortality	Mortality yield (%)	Mortality	Mortality yield (%)
0/3	0	0/3	0

The Based LD_{50} of this fraction was estimated to be 5000mg/kg body weight, according to the OECD Test Guideline [13].

Table 5: The result of the alkaloïd-enriched fraction from *P. africana* aqueous extract

Dose (mg/kg)	*Mortality at 72h*	*Mortality rate at 72h (%)*
2000	2/3	66.66
300	0/3	0

The LD_{50} of the alkaloïd-enriched fraction from *P. africana* aqueous extract was estimated to be 1000 mg/kg body weight according to the OECD Test Scheme [13].

3.5. Gastrointestinal motility test

The result of the effect of *P. africana* aqueous and hydroethanolic extracts on normal intestine transit is summarized in table 6. As indicate in this table, the Loperamide (5 mg/kg/b.w.); *P. africana* aqueous and hydroethanolic extracts at dose of 500mg/kg bw has inhibited respectively the normal intestine transit of 20.184%, 15.36% and 25.84%. Also, acetylcholine and *P. africana* hydroethanolique extract (50 mg/kg bw) increased the normal transit of 9.68% and 3.40% respectively. For *P. africana* aqueous extract, the dose of 50 mg/kg bw, has virtually no effect on normal intestine transit. The highest rate of inhibition and the most important stimulatory activity was

obtained with the hydroethanolic extract in dose dependante manner.

Table 6: Effect of *P. africana* aqueous and hydroethanolic extracts on normal intestine transit

Treatment	*Dose (mg/kg)*	*Rate of total length covered*	*Inhibition rate (%)*	*Stimulation rate (%)*
Normal control		64.17 ± 8.05		
Acétylcholine	0.1	73.84 ± 4.63		9.68
Lopéramide	5	43.98 ± 4.81	20.18	
Aqueous extract	50	65.88 ± 5, 31		1.71
	500	48.81 ± 8.67	15.36	
Hydro-ethanolic extract	50	67.57 ± 10.54		3.40
	500	38.33 ± 6.20	25.84	

Mean and Standard Deviation are presented

As shown in table 7, acetylcholine has increased the intestinal transit of 9.68% when compared to normal control. The aqueous extract of the plant at dose of 50 and 500 mg / kg bw has inhibited intestinal transit induced by acetylcholine of 3.97% and 2.70% respectively. The hydroethanolic extract has increased acetylcholine induced intestine transit by 17.33% and 21.56% respectively at the dose of 50 and 500 mg / kg bw.

Table 7: Effect of *P. africana* extracts on acetylcholine induced intestine transit

Treatment	*Dose (mg/kg)*	*Rate of total length covered (%)*	*Inhibition rate (%)*	*Stimulation rate (%)*
Normal Control		64.17 ± 8.05		
Acetylcholine	0.1	73.84 ± 4.63		9.68
Aqueous extract + Ach	50	60.20 ± 6.32	3.97	
	500	61.47 ± 5.88	2.70	
Hydro-ethanolic extract + Ach	50	81.50 ± 9.78		17.33
	500	85.73 ± 6.02		21.56

Mean and Standard Deviation are presented

4. Discussion

Herbal medicines are often mistakenly considered as non-toxic drugs because they are "natural" and their use is increasing particularly in developing countries. Medicinal plants are known to be an immense source of bioactive molecules. However, these products contain a multitude of bioactive principles that can cause damage. Therefore, it is necessary that all these herbal drugs be subjected to safety tests by the same methods as those used for modern drugs.

Investigations about the chemical composition of *P. africana* that are traditionally used to treat various diseases revealed the presence of secondary metabolites with important pharmacological activities. Indeed, phytochemical screening of aqueous and hydroethanolic extracts of the plant have shown the presence of steroids, triterpenes, anthocyanosides, tannins, saponosides, reducing compounds and carbohydrates in *P. africana* extracts. These results confirm previous studies with differents extracts of differents parts of this plant [6], [15], [16].

Previous studies have shown that terpenes have irritating properties. Terpenes and sterols are toxic to cold-blooded animals. Terpenes act on the nervous system causing incoordination, paralysis and death. They induce digestive disorders, skin and eyes irritations. Alkaloids cause tachycardias, ureters paralysis, decrease of intestinal peristalsis it secretions. Some alkaloids are local anesthetics and induce hyperthermia, mydriasis, constipation, nausea and psychic disorders [17]. Heterosides, on the other hand, increase intestinal motility and inhibit water, sodium and chlorine reabsorption [17].

Tannins impregnate the superficial layers of skin, they have vasoconstrictive properties and are able to bind to proteins, reducing their digestibility [18]. This propertie of tannins are related to their ability to inhibit the metabolic utilization of amino acids after absorption [19]. Thus, the activity of aqueous and hydroethanolic extracts of *P. africana* on gastrointestinal motility is related to the presence of triterpenes, tannins and glycosides in these extracts. Saponins are cytotoxic and have hemolytic properties [20].

World health organization (WHO) recommends that medicinal herbs would be the dominant source to obtain a range of drugs. Therefore, such medicinal plants must be investigated for better understanding their medicinal properties, safety and effectiveness [21]. Safety of plant extract is evaluated mostly by acute oral toxicity analysis. The results of acute toxicity study of *P. africana* extracts showed that the oral administration of the total extracts and the alkaloid-enriched fraction of hydroethanolic decoction did not show any sign of toxicity and any mortality at 2000 mg/kg body weight in mice. The estimated oral LD_{50} for these extracts was 5000 mg/kg. According to the WHO (2010) classification and the OECD Globally Harmonized Classification System [13], these extracts may be classified as Category 5 of substances without acute toxicity effects. This indicated that the oral administration of *P. africaca* leaves total extracts and the alkaloid-enriched fraction of hydroethanolic decoction could be considered safe.

Our results are similar to those of [22] who found an oral LD_{50} greater than 3000 mg/kg in the rat with *P. africana* stem bark ethyl acetate extract. Some authors also showed that *P. africana* stem bark methanolic extract has an oral LD_{50} of 3807.9 mg/kg in mouse and greater than 5000 mg/kg in rat [23].

The estimated LD_{50} of alkaloid-enriched fraction of *P. africana* aqueous extract was 1000mg/kg body weight. This extract could be classified in category 4 of the substances moderately toxic according to United Nations classification [24]. These results are similar to those of [25] who showed that prosopinine, an alkaloid isolated from *P. africana*, had an oral LD_{50} of 820 mg/kg in mice.

From the results of this study, it is hypothesized that extracts of *P. Africana* are relatively safe for use in traditional medicine. However, the aqueous form should be avoided because of the toxicity of it alcaloids.

The study of the effect of *P. africana* extracts on *in vivo* intestinal peristaltism show that at low dose, the aqueous extract of *P. africana* has almost no effect on normal intestinal transit. This extract increased the normal transit of 1, 708 at the dose of 50 mg/kg of body weight.

At a high dose, the aqueous extract of *P. africana* significantly decreased normal intestinal transit with an inhibition rate close to that of loperamide.

The hydroethanolic extract of *P. africana* resulted in a slight increase in normal low-dose transit while at high doses this extract inhibited normal transit better than loperamide, which is used as a positive control.

The traditional use of aqueous forms of *P. africana* in the management of pathologies could cause constipation in the patient, given its high inhibitory power.

Loperamide exerts its antidiarrheal activity by an anti-secretory mechanism. It inhibits intestinal motility by acting directly on the circular muscles and long muscles of the intestine [26]. It could also inhibit the release of acetylcholine and certain prostaglandins in the intestinal wall, which decreases propulsive peristalsis, prolongs the intestinal transit time and increases the absorption capacity of the intestinal wall fluids.

According to [27], loperamide is an opioid receptor agonist and a calcium-calmodulin complex antagonist. It also inhibits the transmembrane calcium flux of certain tissues.

The inhibitory activity of the aqueous and hydroethanolic extracts of *P. africana* could be attributed to its ability to activate opiate receptors or to block calcium channels such as loperamide. It is also possible that these extracts act by an anti-secretory mechanism by activating the Na^+/k^+ ATPase pump [28].

Acetylcholine stimulates intestinal transit at 9.675% at a dose of 0.1 mg/kg. Its activity is inhibited by the aqueous extract of *P. africana*. Indeed, the simultaneous administration of acetylcholine at 0.1 mg/kg and aqueous extract of *P. africana* causes inhibition of intestinal transit induced by acetylcholine. This extract therefore reduces intestinal transit and prevents the action of acetylcholine such as atropine. Indeed, according to [29], atropine reduces intestinal transit through its anticholinergic activity that blocks muscarinic receptors.

Thus, the aqueous extract of *P. africana* inhibits intestinal transit induced by acetylcholine through anticholinergic activity that blocks muscarinic receptors such as atropine or other mechanisms that may lead to inhibition of intracellular calcium mobilization such as inhibition or blocking synthesis of inositol trisphosphate (IP_3) and prostaglandin or by blocking calcium channels.

The inhibitory activity of the aqueous extract of *P. africana* on normal transit is greater than that on transit induced by acetylcholine. Further tests will elucidate the mechanism of action of these extracts.

The hydroethanolic extract of *P. africana* increased intestinal transit induced by acetylcholine. This extract contains substances that potentiate the activity of acetylcholine on gastrointestinal transit.

Phytochemical analysis of the extracts revealed the presence of several chemical groups such as tannins, saponosides, reducing sugars, sterols, and triterpenes.

The spasmolytic activity of the extracts of *P. africana* would be due to the presence of these chemical groups in these extracts. Indeed, these compounds can react on the opioid receptors located on the intestinal smooth muscle and cause the inhibition of intestinal transit [30], [31].

According to [32], coumarins, anthraquinones and alkaloids have purgative properties. However, phytochemical analysis revealed the presence of coumarins and alkaloids in the hydroethanolic extract of *P. africana.* The presence of these compounds could justify the stimulation of gastrointestinal transit induced by acetylcholine.

5. Conclusion

In the present study, phytochemical profile, acute toxicity and effect on gastrointestinal motility of extracts from *Prosopis africana* leaves were investigated. The phytochemical screening revealed the presence of various chemical compounds in *P. Africana* leaves extracts. Acute toxicity studies show that the plant extracts have a low toxicity when they were administrated orally. *Prosopis africana* extracts also interact with intestinal transit either by stimulating or inhibiting it. These results could also explain the many forms of use of this plant in traditional medicine.

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