RESEARCH ARTICLE

TOXICOLOGICAL AND ANTIDIARRHEAL STUDIES OF AQUEOUS EXTRACTS OF TWO MEDICINAL PLANTS: SCLEROCARYA BIRREA (A. RICH) HOCHST (ANACARDIACEAE) AND PSIDIUM GUAJAVALINN (MYRTACEAE) IN NMRI MICE

Sawadogo Touwindséda Aimée, Tougouma Larba Colette, Ouedraogo Youssoufou, Da Filkiène Léonard, Bayala Balé and Belemtougri G. Raymond

Laboratory of Animal Physiology, UFR of Life and Earth Sciences, University Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.

Abstract

Sclerocarya birrea and Psidium guajava are two medicinal plants traditionally used in rural areas for the treatment of many diseases, including diarrhea. In our experiments, we used plants leaves aqueous extract for toxicity evaluation and preventive antidiarrheal effect study in NMRI mice. Sclerocarya birrea and Psidium guajava caused no mortality and no behavioral changes up to a dose of 5000 mg/kg body weight. The extracts of both plants were reported to be practically nontoxic. Diarrhea was censed induced by castor oil consumption, about 4 hours after oral administration in NMRI mice. Then 300, 500 and 800 mg/kg body weight of plant extract was given to animals. Sclerocarya birrea extract induced an antidiarrheal protection of 40, 80 and 100% according to above respective used doses; these values are 60, 80 and 100% respectively with Psidium guajava extract, in same conditions. A one dose test of 300 mg/kg body weight, Psidium guajava extract showed more antidiarrheal effect compared to Sclerocarya birrea. Our work confirms the relevance in the traditional use of both plants in the treatment of diarrhea.

Introduction:

Diarrhea is a disorder of intestinal transit characterized by abundant liquid faeces or with a periodicity of at least three faeces per day (Bryce et al., 2005; Randremanana, 2012). Diarrheal diseases cause about 1.8 million deaths annually worldwide, 90% of which are among children under five years (Cazaban et al., 2005). They are third leading cause of death in pandemics (WHO, 2011; Assogba et al., 2012), the fifth leading cause of early death worldwide (WHO, 2014) and the second leading cause of death in children under 5 years of age (Sidibé, 2014).

A study has shown that in Burkina Faso, precisely in Ouagadougou, diarrheal diseases affect much more young children, with 55.7% of cases among infants (Sidibé, 2014). Because of difficulty to prevent diarrheal diseases (insufficient hygienic conditions in developing countries) curative treatment is prioritized but are too late, many times.

Present study consists in toxicity and preventive antidiarrheal activity of Psidium guajava and Sclerocarya birrea extracts evaluation in NMRI mice.

Corresponding Author: - Sawadogo Touwindséda Aimée
Address: - Laboratory of Animal Physiology, UFR of Life and Earth Sciences, University Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.
Materials and Methods:

Materials:
Biological Material:
Biological material consists of plant material (Sclerocaryabirrea and Psidiumguajava) and laboratory animals.

Plant material:
Sclerocaryabirrea:
The fresh leaves of *Sclerocaryabirrea* were harvested in Gampéla village, 25km from Ouagadougou, in July 2016. The identification was made from the herbarium of the University Joseph KI-ZERBO where a specimen was kept under the number ID 16959 and the sample number 6836. They were dried in the shade under ventilation in the laboratory at room temperature. Once dried, these leaves were ground using an electric grinder to obtain a fine powder which was used for extractions.

![Fig. 1: Sclerocaryabirrea with unripe and ripe fruits.](image1)

Psidiumguajava:
Fresh leaves of *Psidiumguajava*, were collected in the vicinity of University Joseph KI-ZERBO and were treated as those of *Sclerocaryabirrea*. One specimen was kept under ID number 17909 and sample number 6911.

![Fig. 2: Psidiumguajava with a white flower.](image2)
Laboratory Animals:
Naval Medical Research Institute (NMRI) mice was used. The mice were from 8 to 9 weeks-olds and weighing 20-35g respectively. The animals were kept in polycarbonate cages and housed under standard conditions of temperature (22 ± 3˚C), relative humidity (50 ± 10%) and dark/light cycle (12 h/12 h). The animals were given pelleted food containing an average of 29% protein and drinking water ad libitum. Study protocols and ethical issues were approved by Ethical committee of Faculty of University Joseph KI-ZERBO.

Methods:
Aqueous extraction:
The leaves were stored in the Laboratory, protected from dust and humidity, under artificial ventilation and at room temperature. Once dried, the leaves were powdered for the preparation of the various extracts. Test samples of 100 g of the vegetable powder were placed in a 2000 mL stainless steel Erlenmeyer flask. For the aqueous maceration, a volume of 1000 mL of distilled water was added to each test sample and homogenized with a glass rod. After homogenization, the mixture was kept under constant mechanical stirring for 24 hours at laboratory room temperature. After this, the mixture was filtered twice, successively on a fine nylon fabric and then on cotton wool. The filtrate (aqueous macerate) obtained was distributed in 500 mL crystallizers and placed in a ventilated oven (MEMERT) set at 45°C for 24 hours. The concentrated extracts obtained were transferred to freezing bottles and used for the various tests. The yield of the aqueous extraction was 12.14% Sclerocaryabirrea and 12.26% Psidiumguajava.

Toxicity study:
Acute toxicity:
The test was conducted according to OCDE (2001) guideline 423. It was performed with nine female mice, weighed and labelled and then divided into three groups of three mice. The control group received distilled water. Groups two and three received Sclerocaryabirrea and Psidiumguajava extract at 5000 mg/kg of body weight (BW), respectively. Twenty-four (24) hours prior administration of the extract, the mice were fasted and four (04) hours prior administration, they were deprived of drinking water. Administration is made in a single dose by oral route. Observations are made after 1 hour, 24 hours, 48 hours, 72 hours and for 14 days after extract administration. One hour after extract administration, mice are re-supplied with water and food. During this period, changes in behavior are noted as well as the number of dead animals.

Subacute toxicity:
Subacute toxicity was conducted according to the OCDE (2008) guideline 407. Female mice were used during subacute toxicity. They were divided into four (04) groups of six (06) mice each. Doses of 300, 400 and 800 mg/kg body weight of Sclerocaryabirrea and Psidiumguajava were administered. NaCl 0.9% was administered to the control groups. The test substance was daily administered, at the same time, over a period of 28 days, by single dose gavage in 1 mL/100g of body weight. Behavioral changes of the mice were noted during the experiment. Animals’ weight and food consumption were weekly recorded during the test.

Antidiarrheal test:
The antidiarrheal test was performed according to the method of Shah et al. (2011a) with slight modifications. Twenty-five (25) female mice of eight to ten (8-10) week-old were used. The animals were fed 24 hours before the experiment and deprived of water 10 hours before.

Preliminary studies:
During the preliminary study the mice were divided into 5 groups of 5 mice each. All experimental mice received castor oil (10 ml/kg of BW) orally. Four hours (4 h) after castor oil administration, each mouse is placed in an individual cage containing blotting paper, and presence or absence of diarrheal faeces was observed.

All mice that did not have diarrhea were removed and those that did have diarrhea were collected and put in standard conditions for one week.

Antidiarrheal studies:
The mice were divided into 5 groups of 5 mice each:
Group 1 received NaCl (0.9%) (negative control);
Group 2 received 300 mg/kg BW of the extract;
Group 3 received 500 mg/kg BW of the extract;  
Group 4 received 800 mg/kg BW of the extract;  
Group 5 received 10 mg/kg BW of loperamide (positive control).  
One hour (1 h) after treatment, each mouse received 10 mg/kg BW of castor oil and 4 h after, observations were done as in above preliminary studies.  
The substances were administered orally.

**Results and Discussion:**

**Results:**

**Aqueous extract:**  
The aqueous extract obtained was in brown crystalline form and the yield was 12.14% for *Sclerocaryabarrea* and 12.26% for *Psidiumguajava*.

**Acute toxicity:**  
The dose of 5000 mg/kg body weight was used. Three female animals were used at each dose level. No mortality was observed in either plant.

**Subacute toxicity:**  
Results are plotted in figure 3. Doses of 200, 400 and 800 mg/kg BW of *Psidiumguajava* administered resulted in a slight non-significant weight loss over the four weeks of the study. The same doses of *Sclerocaryabarrea* administered resulted in non-significant weight loss during the first week. However, a change in weight was recorded during the last three weeks.

![Fig. 3: Weight evolution with *Psidiumguajava*.](image-url)
Antidiarrheal test:
Sclerocaryabirrea extract and Psidiumguajava extract at doses of 300, 500 and 800 mg/kg significantly reduced castor oil induced diarrhea compared to the negative control.

Psidium guajava antidiarrheal test

Fig. 4: Weight evolution with Sclerocaryabirrea.

Fig. 5: Percentage Protection with Psidiumguajava.
Fig. 6: Percentage protection with *Sclerocarya birrea*.

**Discussion:**

The purpose of this study was to evaluate the toxicity and effect of aqueous extracts of *Sclerocarya birrea* and *Psidium guajava* in the treatment of castor oil induced diarrhea.

The leaves of *Sclerocarya birrea* and *Psidium guajava* were used to make the aqueous extract in our study. Indeed, studies carried out by authors such as Gueye et al. (2012), Diatta et al. (2013) have shown that the leaves are the parts of plants most used in traditional medicine in the management of several pathologies. Their uses are known and justified as the place of synthesis of secondary plant metabolites and by the quantity of chemical compounds they contain (Lumbu et al., 2005; Mangambu et al., 2014). These preparations are used as or in beverages. This use can be justified by the fact that diarrhea is related to bacterial, fungal and/or parasitic affections located in deep organs. For the treated ones, any substance must reach the digestive tract to facilitate absorption (Tra Bi et al., 2008) and treatment. This justifies the use of the leaves of our two plants and their oral administration.

The study of the toxicity of *Sclerocarya birrea* and *Psidium guajava* for medical use allowed us to determine their safety for use without risk of intoxication. In this study, the acute toxicities of *Sclerocarya birrea* and *Psidium guajava* indicated a LD$_{50} > 5000$ mg/kg BW. According to the Globally Harmonized System of Classification (GHS) of the OECD (2001) these two plants are classified as category 5, practically nontoxic. Studies of Galvez et al. (1991) showed that the decoction of *Sclerocarya birrea* bark administered orally has a LD$_{50}$ of 16.44 ± 5.44 g/kg. Mittal et al. (2010) showed that the LD$_{50}$ of the aqueous extract of *Psidium guajava* is greater than 5 g/kg BW. Our data confirm that *Sclerocarya birrea* and *Psidium guajava* are practically nontoxic. This work also demonstrated long-term safety of use during the subacute toxicity study of *Sclerocarya birrea* where mortalities were noted at a dose of 200 mg/kg BW. The toxicity of *Sclerocarya birrea* is a function of product concentration and duration of treatment.

*Sclerocarya birrea* extract administered at doses of 300, 400 and 800 mg/kg BW has a percentage of protection of 40, 80 and 100% respectively against castor oil induced diarrhea. The extract of *Psidium guajava* administered at the same doses has a protection percentage of 60, 80 and 100% respectively. The loperamide used as a positive control has a protection percentage of 100%. The results obtained showed that the aqueous extracts of *Sclerocarya birrea* and *Psidium guajava* significantly reduced castor oil induced diarrhea. However, the 0.9% NaCl used had no effect on diarrhea. Castor oil is a purgative substance. It modifies the intestinal hydro-electrolytic exchanges and stimulates intestinal motility. Indeed, castor oil in the lumen of the small intestine is metabolized into ricinoleic acid which stimulates the secretion of prostaglandins and then reverses the results in stimulating secretion. On the other side this ricinoleic acid induces the release of certain mediators from the intestinal tract such as histamine, nitric oxide and prostaglandins which, in turn, stimulate intestinal secretion, motility, permeability, and prevent the reabsorption of sodium, potassium, and water (Belemtougri et al., 2016).
The antidiarrheal action of aqueous extracts may be due to the inhibition of the increase in water secretion that occurs in all acute diarrhea. Similar results with a percentage of protection of 40 and 60% at the respective doses of 300 and 500 mg/kg BW were obtained by Belemtougri et al. (2016) with extract of Sclerocaryabirrea on castor oil induced diarrhea.

Phytochemical studies carried out on a few plants indicate the presence of tannins, flavonoids, polyphenols, polyterpenoids, alkaloids and saponins, all extractable by water. Biswas et al. (2013) showed that the aqueous extract from the leaves of Psidiumguajava contains tannins, polyphenols, triterpenes, flavonoids, essential oil, saponosides, alkaloids and carotenoids. Similarly, the phytochemical screening carried out by Belemtougri et al. (2006) indicated the presence of tannins in the aqueous extract of Psidiumguajava leaves and the presence of tannins, anthocyanins, alkaloids, flavonoids and triterpenes in the ethanolic extract. Nacouma/Ouedraogo (1996) and Belemtougri et al. (2007) noted the presence of flavonoids, tannins, catechins, procyanidins, anthocyanins and phytosterols in the leaves of Sclerocaryabirrea. The presence of these chemical compounds could be at the origin of the antidiarrheal activity of these two plants as these chemical compounds are known for their antimicrobial and antidiarrheal properties (Sérémé et al., 2008; Tra Bi et al., 2008). Galvez et al. (1991) also found similar results using methanolic extract from the bark of Psidiumguajava rich in tannin in the treatment of diarrhea. A study of Nicolas (2012) also showed that the decoctions of Psidiumguayava and Euphorbia hirta are used in the treatment of diarrhea.

By comparing our results with those obtained previously, we can confirm the antidiarrheal effect of aqueous extracts of Sclerocaryabirrea and Psidiumguajava leaves. Our work shows that Psidiumguajava leaves extracts are more active than Sclerocaryabirrea extracts at a dose of 300 mg/kg BW.

**Conclusion and Perspectives:**

This study assessed the toxicity of two medicinal plants used in the traditional antidiarrheal treatment. The method of preparation used is maceration and the administration was done orally. The effects induced by Sclerocaryabirrea and Psidiumguajava, to treat diarrhea, seem to be due to various chemical groups: alkaloids, flavonoids, tannins and polyphenols that form the scientific basis for the traditional therapeutic use of these plants. The study of the acute toxicity of Sclerocaryabirrea and Psidiumguajava indicates that the extracts would be practically nontoxic at the doses used. On the other hand, the use of Sclerocaryabirrea over a long period of time should be done with caution.

From these results the following perspectives are considered:

1. Sub-chronic oral toxicity of Psidiumguajava and Sclerocaryabirrea followed by the determination of hematological and biochemical parameters;
2. Phytochemical screening of Sclerocaryabirrea and Psidiumguajava for the detection of chemical compounds such as flavonoids, tannins, alkaloids by thin layer chromatography;
3. Studies on the isolated intestine to determine the mechanisms of action of Sclerocaryabirrea and Psidiumguajava extracts;
4. Testing for antimicrobial activity with extracts of Sclerocaryabirrea and Psidiumguajava;
5. Studies on other models of diarrhea such as fluid accumulation and electrolyte secretion and gastrointestinal transit assay.

**References:**