Toxicological Effects of *Sclerocarya birrea* (A. Rich) Hochst (Anacardiaceae) and *Psidium guajava* L. (Myrtaceae) Leaf Extracts on Mice and Their Pharmacological Effects on Rat Duodenum

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**Abstract:** The aim of the present study was to investigate the toxicological effects of *Sclerocarya birrea* and *Psidium guajava* leaf extracts on mice and the antispasmodic effect on rat duodenum. The two plants extracts showed toxicological effects on mice in dose dependent manner. The antispasmodic effect was conducted as follow: In the first part of experiments, cumulative dose-response curve for Ach was obtained and then dose-response curves are repeated after addition of atropine and different doses of the extracts. In the second part, extracts were applied to the tissue after contraction with Ach. The extracts have exhibited an inhibitory effect on the dose-response curves induced by Ach on rat duodenum and significantly reduced the maximal response of Ach in a concentration-dependent manner. These results can partly justify the use of these plants in traditional medicine.

**Key words:** *Psidium guajava*, *Sclerocarya birrea*, rat duodenum, ach, relaxation, antispasmodic

**INTRODUCTION**

*Psidium guajava* (Myrtaceae) is native of tropical America and has been naturalized in tropical countries of the world. All the parts of the plant are used by healers to treat various human ailments such as cough, wounds, ulcers, bronchitis, eye sores, bowels, diarrhoea, cholera and other gastro-intestinal disorders.

A broad spectrum of biological and pharmacological effects of extracts *Psidium* leaves have been reported such as antinociception (Santos et al., 1998; Shaheen et al., 2000), CNS depressor (Meekes et al., 1996), antimitogenic (Matsuo et al., 1996; Grover and Bala, 1993), antihyperglycemic (Obatomi et al., 1994), anti diarrhoeic (Lutterodt, 1989), antibiotic (Lutterodt, 1989; LeGrand, 1989), antiamoebic and antispasmodic (Tona et al., 2000; Lozoya et al., 2002), antitussive (Jairaj et al., 1999) and narcotic-like activity (Lutterodt and Maleque, 1988). Microbicidal (Vieira et al., 2001), inotropic (Garcia et al., 2003) and antioxidant (Qian and Nihoirimbere, 2004) effects have also been studied.

*Sclerocarya birrea* is a tree widely distributed from Subsaharian to southern Africa. In southern Africa, it is called Marula by indigenous people (Eloff, 2001) and is considered as power plant (Balick, 1990). It is widely used to treat many affections and diseases such as hypertension, dysentery, diarrhoea and gastro-intestinal diseases. The leaves and bark decoctions are used to treat diabetic patients (Nacouma-Ouedraogo, 1996). Bark and leaves showed antibacterial activity (Eloff, 2001).

Secretagogue (Galvez et al., 1992), anti-inflammatory (Ojewole, 2003a), antioxidant (Braca et al., 2003) and hypoglycemic (Ojewole, 2003b) activities have been reported.

Chemical screening showed mainly tannins (Bonafo-Kouda, 1998) and flavonoids and tannins (Belentougri et al., 2001), in leaf extract. A new flavonol glycoside, quercetin 3-O-alpha-L-(5''-galloyl)-arabinofuranoside, phenolic compounds and epicatechin derivatives (Braca et al., 2003) are isolated from leaves.

Pharmacological studies showed that bark extract had antidiarrhoeal activity (Galvez et al., 1993), leaf extracts inhibited angiotensin converting enzyme (Duncan et al., 1999) and decreased internal calcium concentration in cultured rat skeletal muscle cells (Belentougri et al., 2001).

To date, no studies have been performed about the toxicological potential of the two plants.

The present study describes the toxicological effects in mice and antispasmodic effects of *Psidium* and *Sclerocarya* leaf extracts in rat duodenum. This could
contribute to the understanding of the mechanism underlying their actions and their uses in traditional medicine.

MATERIALS AND METHODS

Plants collection: Fresh leaves of *Sclerocarya birrea* were collected from Campêla (Burkina Faso, West Africa) in July 2001 whereas fresh leaves of *Psidium guajava* were collected around Ouagadougou in the same period. These plants were identified by Pr. Millogo-Rasolodimby, Department of Botany, University of Ouagadougou. The herbarium specimens have been deposited in this Department.

Preparation of plants extracts: Crude decoctions were prepared from the shade dried leaves. Twenty five grams of leaf powder of each plant were macerated in 1 L of deionized water for 24 h at room temperature and then boiled for 10 min to mimic the traditional preparation methods. After cooling, the resulting extract was filtered through Whatman No. 2 and freeze-dried for 24 h and then lyophilized to give brown powder which was utilized for experiments.

Animals: NMRI (Naval Medical Research Institute) adult mice (20-30 g) and Wistar rats (180-250 g) were used in these studies. The animals were fed with standard diet and were kept at 24±2°C and submitted to a 12 h light/dark cycle with free access to food and water. Twelve hours before experimentation, the food was removed but water remained available *ad libitum*.

All animal procedures were strictly within national laws and guidelines.

Acute toxicity assessment: NMRI mice of both sexes with a weight range of 20-30 g were used in this investigation. Doses of *Sclerocarya* leaf extract (300, 700, 1000, 1500 and 2000 mg kg⁻¹) were administered i.p. to five groups of mice. Different doses of *Psidium* leaf extract (845, 1080, 1500, 2000 and 3000 mg kg⁻¹) were also administrated to another five groups of mice. Control groups received an equivalent dose of the vehicle. Test and control groups were observed for 48 h under normal environmental conditions with free access to food and water.

Antispasmodic study: Wistar rats of either sex were used for this experiment. They were killed by cervical dislocation. A portion of the duodenum was removed and placed in Tyrode's solution at room temperature. The connective tissue was carefully dissected from the tissue and then suspended in a 20 mL organ bath containing Tyrode's solution of the following composition (mM): NaCl, 136.7; KCl, 2.69; CaCl₂, 1.8; MgCl₂, 0.1; NaH₂PO₄, 0.04; NaHCO₃, 11.9 and Glucose, 5.5 and adjusted at pH 7.4 with Tris. The solution was continuously maintained at 37°C and aerated by air. A load of 1 g was applied and a 60 min equilibration period was allowed, during which the physiological solution was changed every 10 min to protect against interfering metabolites (Altura and Altura, 1970). At the end of the equilibration period, acetylcholine (Ach) (10⁻⁴M) was applied to assess the tissue viability. The extracts were added directly to the organ bath in volumes not exceeding 5% of bath volume. The blocking effect of atropine and extracts were investigated on Ach induced contractions of the duodenum using a 10 min contact. The relaxation was carried out with cumulative additions of the extracts after Ach induced persistent contractions. Isotonic contracts were recorded using Harvard isotonic transducer and displayed on a Harvard Student Oscillograph pen recorder device.

Drugs: Drugs used were acetylcholine chloride (Ach) and atropine sulphate (Sigma Chemical Company, USA). All drug solution was freshly prepared.

Statistical analysis: Results were expressed as mean±SEM and unpaired students t-test was used to test for significant difference between the means. Statistical analysis of the data was done using Graph Pad Prism version 3.0 (Graph Pad software, San Diego, CA, USA). p<0.05 was considered as significance. Concentration-response curves were analysed by non-linear regression (Graph Pad Prism).

RESULTS

Acute toxicity of the extracts: To establish the safety, different doses of *Sclerocarya* and *Psidium* were administered to both sexes of mice. The extracts exhibited toxicity in dose dependent manner. *Sclerocarya* at 300 and 700 mg kg⁻¹ i.p. showed no lethal effect, while 1000, 1500 and 2000 mg kg⁻¹ exhibited 40, 60 and 100% mortality, respectively.

*Psidium* exhibited no lethal effect at 845 and 1080 mg kg⁻¹, while 1500, 2000 and 3000 mg kg⁻¹ showed 60, 80 and 100% lethal effect, respectively. Abdominal contractions and difficulties in locomotion and breath were observed immediately in treated groups after the extract injection and for 2 h after. The LD₅₀ was 850 mg kg⁻¹ for *Sclerocarya* and 1250 mg kg⁻¹ for *Psidium*.
According to Hodge and Sterner (1943), *Sclerocarya* was moderately toxic and *Psidium* slightly toxic.

**Antispasmodic effect:** The aqueous extracts of *Sclerocarya* and *Psidium* failed to display activity on rat duodenum basal tone when applied to the organ bath. Then we utilize Ach a well known spasmoden on rat duodenum to research some antagonistic effect.

Acetylcholine (Ach) caused concentration-dependent contraction of duodenum, reaching their maxima within 40s of contact. *Sclerocarya birrea* in a dose dependent manner inhibited the duodenum contraction induced by Ach with pD<sub>2</sub> values of 6.20±0.10 (12.5 µg mL<sup>-1</sup>), 6.01±0.02 (50 µg mL<sup>-1</sup>) and 6.05±0.10 (100 µg mL<sup>-1</sup>) (Fig. 1). *Psidium guajava* also inhibited the tension developed by Ach in a dose dependent manner with pD<sub>2</sub> values of 4.03±0.42 (50 µg mL<sup>-1</sup>) and 4.09±0.37 (100 µg mL<sup>-1</sup>) (Fig. 2).

It is interesting to observe that at the same dose (100 µg mL<sup>-1</sup>), extract of *Psidium* is more active than that of *Sclerocarya*.

The shift of the contraction curves to Ach was observed with extract of *Psidium* in a dose dependent manner.

![Fig. 1: Cumulative log dose-response curves on the rat duodenum to acetylcholine after preincubation for 10 min with *Sclerocarya birrea* leaf aqueous extract at indicated concentrations, V 12.5 µg mL<sup>-1</sup>; ○ 50 µg mL<sup>-1</sup>; ◇ 100 µg mL<sup>-1</sup>; Ach. *p<0.05](image1)

![Fig. 2: Cumulative log dose-response curves on the rat duodenum to acetylcholine after preincubation for 10 min with *Psidium guajava* leaf aqueous extract at indicated concentrations, ● 50 µg mL<sup>-1</sup>; ○ 100 µg mL<sup>-1</sup>; ◇ atropine (10<sup>-7</sup> M); △ Ach. *p<0.05; **p<0.01; ***p<0.001](image2)

In another set of experiments, the effects of *Sclerocarya* and *Psidium* extracts were tested by adding the extracts into the organ bath containing Ach (10<sup>-7</sup> M) when the contraction reached a plateau. Under this condition, *Sclerocarya* and *Psidium* extracts produced a rapid and dose dependent manner relaxation of the contraction developed by Ach (Fig. 3 and 4). At 100 µg mL<sup>-1</sup>, *Sclerocarya* relaxed the contraction by 50% (Fig. 3), while *Psidium* relaxed the contraction by 85% (Fig. 4). These effects were observed with 5 min after the addition of the extracts into the bathing solution.

These inhibitions were significantly larger than that observed when the duodenum was pre-incubated for 10 min in the presence of *Sclerocarya* and *Psidium* extracts before inducing the contractions.

Considering the effectiveness of the different extracts, we can conclude that the extract of *Psidium* was more active than that of *Sclerocarya*. 

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The acute toxicity test showed that the two plants could be classified in the group of slightly toxic substance. Our results are low that those found by Cjewole (2003b) in aqueous and methanolic extracts of Sclerocarya stem bark. This difference can be explained by the nature of the components present in the extracts, by the mode of administration and also by the parts of the plant used. Indeed, several investigators have demonstrated that LD₅₀ obtained by oral administration is high than that obtained by intraperitoneal administration. The death of animals which have received high doses may be due to metabolic effects of metabolite imbalance or some kind of nervous toxicity.

Our results showed that the two plants extracts inhibited the contractions induced by Ach on rat duodenal smooth muscle in dose-dependent manner. The extract of Psidium was more active than that of Sclerocarya. The difference could be due to the chemical nature of the components.

The inhibitory responses observed attenuated Ach responses, a known muscarinic agonist, suggesting that cholinergic mechanisms may be involved in the observed effects. Anticholinergic agents like atropine are known to block muscarinic receptors, thereby causing relaxation in spontaneous contractions of smooth muscles (John and Palmer, 1995).

It has been reported that Ach could increase the activity of membrane bound phospholipase C enzymes and generation of inositol triphosphate (IP₃) (Sadraei et al., 2003). IP₃ released in the cytoplasm will interact with the receptor on intracellular Ca²⁺ store sites and causes the release of intracellular Ca²⁺ stores. Therefore, our extracts can act in this way by blocking a cascade of reactions which lead to calcium release and then inhibited the contractions induced by Ach.

The observed results showed that a high dose of Psidium inhibited more the contraction induced by Ach than that of atropine. Taken together this observation suggests the Psidium extract contains more than one compound. Indeed, more than twenty identified compounds from Psidium guajava leaf have been reported in the literature (Seshadri and Vasishta, 1965; Osman et al., 1974; Lutterodt and Maleque, 1988). It has been reported that the leaf extract main component identified as quercetin has effect on the intracellular calcium levels in gastro-intestinal smooth muscle (Lutterodt, 1989; Lozoya et al., 1990). It has been reported that quercetin had many pharmacological activities. Formica and Regelson (1995) reported that quercetin exhibited spasmylytic effect. It has relaxant effect on vascular and intestinal smooth muscle (Duarte et al., 1993; Morales et al., 1994).
According to Re et al. (1999), it reduces acetylcholine release in neuromuscular junctions due to its interactions with calcium channels of presynaptic membranes. It also antagonizes the inward calcium membrane current leading to a decrease of smooth muscle contractile force (Lozoya et al., 1994). It caused relaxation of the rat isolated ileum in concentration dependent manner (Hammad and Abdalla, 1997).

These biological activities of quercetin showed that flavonoids could play an important role in the relaxation of rat duodenal smooth muscle in our assays.

On the other hand, tannins which exist in the extract could also play an important role. It has been reported that it has spasmolytic activity in smooth muscle cells (Tona et al., 1999).

Few studies have been reported on Sclerocarya birrea biological activities. Antidiarrheal activity and spasmolytic effect on guinea-pig ileum has been reported (Galvez et al., 1993). These effects seemed due to the procyamin isolated from the bark.

CONCLUSIONS

The results reported here show for the first time the toxicological effects of the two plants in mice. These results showed that aqueous extracts have spasmolytic effects in dose-dependent manner and could explain their traditional use as antidiarrheal substances. Additional investigations are needed on the chemical characterization of the active principle(s) which cause relaxation on mammalian smooth muscles.

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REFERENCES


