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Curative Dose of *Khaya grandifoliola* Stem Bark for the Treatment of Gastric Ulcers Using Wistar Rats

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Abstract

Optimum therapeutic doses of the aqueous extract of *Khaya grandifoliola* (Welw) C.D.C. stem bark, used in traditional medicine for the treatment of gastric ulcers, have been determined. In order to specify the highest dose that can be administered without producing toxic effects, the LD_{50} of this plant drug was investigated and found to be 5.5 g/kg body weight. This can be compared with a minimal inhibitory concentration (MIC) of 12.4 mg/kg body weight, which gives 100% inhibition of gastric ulcers in stress-ulcerated rats. The average traditional medical practitioner's (TMP) dose of 8.4 mg/kg body weight gave 89.3% inhibition, which was the same as an average dose of 0.285 mg/kg body weight of Pepdine (famotidine), a known pharmaceutical anti-ulcer drug used as control.

Keywords: Gastric ulcers, *Khaya grandifoliola* (Welw) C.D.C. stem bark, LD_{50}, Meliaceae, therapeutic dose.

Introduction

Khaya grandifoliola (Welw) C.D.C. (Meliaceae) stem bark is used in the form of decoctions by traditional healers in West and Central Africa for the treatment of varying ailments (Dalziel, 1948; Metcalf & Chalk, 1957; Agbedahunsi et al., 1998). In that herbal medicines have been used for thousands of years and the World Health Organization (WHO) has recognized the contribution and value of herbal medicines used by a large segment of the world population (WHO, 2002), a considerable number of widely used medicinal plants have been implicated as possible causes of certain long-term disease manifestations (Schoental, 1972; Addae-Mensah, 1992; Sofowora, 1993). In addition, the absence of standard dosages that can lead to overdosage or underdosage has been advanced as an argument against the use of herbal treatment (Addae-Mensah, 1992; Sofowora, 1993).

Efforts are being made in Africa toward establishing scientific evidence for the efficacy claimed for medicinal plants used in traditional medicine (TM) with a view to incorporating the efficacious ones into health care systems and discarding toxic ones so that drug production can be encouraged in African countries. Toward this end, the safety and therapeutic dose of the water extract of *Khaya grandifoliola* (Welw) C.D.C. (Meliaceae) stem bark used in TM (in West Africa) for the treatment of gastric ulcers has been tested in rats.

Materials and Methods

Plant material

Khaya grandifoliola (Welw) C.D.C. (Meliaceae) is a large buttressed tree of up to 45 m in height and 150 cm in girth (Irvine, 1961). Commonly known as African mahogany, this plant is found in the fringe between the rain forest and savannah. It is found throughout western tropical Africa from the Guinea coast through Cameroon and extending eastward through the Congo basin to Uganda and parts of Sudan (Irvine, 1961). In Cameroon, it is found in the forests of Adamawa, Foumban, Meiganga, Yoko, Tibati, Belabo, and Wum (Vivien & Faure, 1985). *Khaya grandifoliola* is easily distinguished from other "acajous" by its large leaflets with numerous secondary veins and large lignus fruits. The young leaves are bright-red and clearly visible at a distance. The bark

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has a bitter taste. The stem bark of *Khaya grandifoliola* was collected from Mankim, 40 km from Foumban in West Cameroon in June 1995 and identified by Professor Amougou Akoa of the Botany Department of the University of Yaounde 1. Voucher specimens (PM 098/95) were deposited in the National Herbarium in Yaounde.

Establishment of therapeutic dose

Experimental animals

Adults Wistar rats weighing 180–230 g were used. The rats were raised in the Animal House of the Faculty of Science of the University of Yaounde 1. They were maintained in a standard environment and fed standard diet. At 48 h prior to experimentation, they were deprived of food but given water *ad libitum*. During this time, they were kept in cages with raised wire mesh to prevent coprophagy and to ensure empty stomachs during experiment.

Stress-induced gastric lesions

Gastric mucosal lesions were induced by stress using the induction method of Takagi and Okabe (1968). After depriving the animals of food for 48 h, the test rats were given varying doses of the plant extract orally, then immediately put into cylindrical cages and immersed in water to the level of the ziphoid. The rats were stressed for 17 h then sacrificed under chloroform anesthesia. The stomachs were opened and the gastric damage observed. The lengths and widths of each lesion were measured. The total surface area covered by the lesions of each stomach was determined by summing up the areas of all the lesions. This was taken as the ulcer index. Percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosal surface area.

Choice of dose of plant extract

To determine the test doses of the plant extract, we referred to the dose used by the traditional medical practitioner (TMP). The TMP boils a ''handful'' of the powdered dry bark in water for an adult patient to drink a glassful morning and evening.

To obtain an equivalent dose to the TMP's dose, an average of handfuls gave us 50 g and the quantity of water averaged 3 l. The decoction of 50 g in 3 l of water was filtered in a no. 3 Whatman filter paper and then lypholized to give an average of 7.080 g and a concentration of 2.36 g/l. An average glass of 250 ml contains 590 mg consumed by an average 70-kg adult. This corresponded to a dose of 8.43 mg/kg taken twice a day.

Testing of the curative effects

With 8.43 mg/kg as an average dose; doses of 1-unit intervals were selected for a dose-response study. Doses of 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, and 13 mg/kg were given to stress-induced ulcerated rats and the percentage ulceration calculated.

Test for safety and establishment of an LD_{50}

Experimental animals

White mice obtained from the Institute Pasteur in Yaounde, aged 8–10 weeks and weighing 26.9–36.3 g, were used. The mice were fed a standard diet and kept for a week for acclimation before experimentation.

The day before the start of the experiment, the mice were starved and repartitioned into 13 groups of 5 mice/group of similar weights. The mice received increasing doses of the plant extract orally; all the mice of a said group received the same dose on a single administration.

Preparation of plant extract and choice of dose

As described above for the establishment of the therapeutic dose, we referred to the dose used by the TMP. The lyophilized powder from the aqueous extract of the *Khaya grandifoliola* plant material was used. Guided by a previously calculated TMP average dose of 8.4 mg/kg, up to now shown nontoxic, 8 mg/kg body weight was used as minimum dose. The first subgroup of mice was given increasing doses in geometric progressions of 2 (i.e., 0.08, 0.16, 0.32, 0.64, 1.28, and 2.56 mg/kg). The second subgroup received increasing doses in arithmetical progression of 1 (i.e., 3, 4, 5, 6, 7, and 8 g/kg). The 13th group received distilled water as control.

Two hours after administration of the drug, the animals were returned to their normal feeding habits. They were put under observation for 24 h followed by a 7-day follow-up. Signs and symptoms of intoxication were observed and registered for each dose. Deaths were registered and percentage mortality calculated. All dead mice were immediately dissected and internal organs examined. All surviving mice at the end of experiment were weighed, sacrificed, and their internal organs examined. The LD_{50} was determined using Kabar's method of calculation as described by Kamanyi (1984).

Results

The results obtained for establishing the therapeutic dose by analyzing the effects of increasing doses of Khaya on stress-induced gastric lesions in rats are given in Table 1. Table 2 gives the comparative results using famotidine as a control drug. Reactions of mice to the increasing doses of *Khaya grandifoliola* water extract are given in Table 3. The LD_{50} was determined to be 5.5 g/kg and the LD_{100} to be 8 g/kg.

Table 1. Effects of increasing doses of *Khaya grandifoliola* on stress-induced gastric lesions in the rat.

Treatment	Dose (mg/kg)	n	Mean ulcer index ± SEM (mm^2)	Percentage ulcerated surface	% Inhibition
Control	—	6	195.6 ± 23.6	16.3	—
Khaya	5.4	6	94.8 ± 3.7	9	51.5
grandifoliola	6.4	6	68.9 ± 3.6	6.5	64.8
extract	7.4	6	41.5 ± 6.4	3.5	78.8
	8.4	6	20.9 ± 1.4	1.7	89.3
	9.4	6	5.4 ± 2	0.5	97.3
	10.4	6	3.3 ± 1	0.3	98.4
	11.4	6	0.6 ± 0.4	0.1	99.7
	12.4	6	0.0 ± 0.0	0.0	100
	13.4	6	0.0	0.0	100

Mean values ± SEM. $p < 0.01$ vs. control, Student's *t*-test. Normal unstressed rats receiving no treatment had no ulcers.

Table 2. Effects of increasing doses of famotidine as control on stress-induced gastric lesions in the rats.

Treatment	Dose (mg/kg)	n	Mean ulcer index ± SEM (mm^2)	Percentage ulcerated surface	% Inhibition
Famotidine	0.135	6	101.5 ± 6.1	7.3	48.1
	0.185	6	78.3 ± 5.2	5.6	60
	0.235	6	48.5 ± 6.4	4.3	75.2
	0.285	6	21.0 ± 1.6	1.5	89.3
	0.335	6	9.9 ± 2.4	0.8	95
	0.385	6	1.0 ± 0.3	0.09	99.5
	0.435	6	0.4 ± 0.2	0.03	99.8
	0.485	6	0.0 ± 0.0	0.00	100

Mean values ± SEM. $p < 0.01$ vs. control, Student's *t*-test. Normal unstressed rats receiving no treatment had no ulcers.

Discussion and Conclusions

A major drawback in the use of plant medicine in African traditional medicine is the often imprecise dosage prescriptions such as "handful," "cupful," "glassful." Such prescriptions can lead to over- or underdosage, as there are variations in handfuls, cupfuls, glassfuls, and so forth. There is, therefore, need to establish the optimum doses of these herbal drugs after establishing proofs for claims of efficacy and safety.

Analysis of the effects of increasing doses of water extracts of the *Khaya grandifoliola* stem bark on stress-induced gastric lesions in rats show that 12.4 mg/kg gives

Table 3. Reactions of mice to increasing doses of *Khaya grandifoliola* extract.

Activity expressed		Dose in g											
		0.08	0.16	0.32	0.64	1.28	2.56	3	4	5	6	7	8
Locomotion	Increased												
	Decreased						+	+	+	+	+	+	+
Bizarre reaction	In rounds				+	+	+	+	+	+	+	+	+
	Backwards			+	+	+	+	+	+	+	+	+	+
Sensitivity to:													
Touch	Increased												
	Reduced						+	+	+	+	+	+	+
Sound	Increased												
	Reduced						+	+	+	+	+	+	+
Exploratory behavior	Increased												
	Reduced						+	+	+	+	+	+	+
Aggressive behavior	Increased												
	Reduced						+	+	+	+	+	+	+
Abnormal tail	Rigid												
	Placid							+	+	+	+	+	+
Convulsions									+	+	+	+	+
Irritations in the eyes:													
Conjuntivities									+	+	+	+	+
Swollen eyes									+	+	+	+	+
Production of tears									+	+	+	+	+
Size; weight loss								+	+	+	+	+	+
Shivering										+	+	+	+
Stool (Diarrhoea)							+	+	+	+	+	+	+
Respiration	Increased						+	+	+	+	+	+	+
	Reduced												

+ = Positive reaction.

100% inhibition. These effects, compared with the effects of varying doses of famotidine, an antiulcer pharmaceutical product used as control drug, on similarly stress-induced gastric ulcers in rats reveal that the mean control drug dose of 0.285 mg/kg gives the same effect as the average TMP's prescription dose of 8.4 mg/kg of *Khaya grandifoliola*. The effect was shown by the same percentage inhibition of 89.3%.

For the calculation of the ulcer index, we used the sum of the areas of the ulcers as this is more indicative of the extent of ulceration, more so than the sum of the lengths of ulcers as the ulcer index proposed by Bishayee and Chatterjee (1994).

In order to specify the highest dose that can be administered without producing toxic effects, increasing doses of *Khaya grandifoliola* water extracts were given orally to mice. Observations made on signs of intoxication upon ingestion of increasing doses show that below 2.56 g/kg body weight, *Khaya grandifoliola* is well tolerated. At higher doses, there were signs of fatigue, prostration, accelerated respiration rate, decrease in locomotion, sensibility to touch and sound, as well as exploration and aggressivity. At doses of >4 g/kg, there were signs of irritation in the eyes, shivering, and convulsion.

Casarett (1968) gave an outline of types of animal toxicology tests. These tests include acute tests, involving single doses; prolonged (subacute) tests, involving daily doses for duration of 3 months; chronic tests, involving daily doses for duration of 2–7 years. Acute toxicity studies are critical because they give a rough idea about the nature of the candidate medicinal plant in addition to determining safe levels for clinical use.

It should be emphasized that definitive toxicology studies are very time consuming and costly. Thus, relatively inexpensive short-term screening procedures are usually employed to identify the most toxic extracts. The exposure of relevant experimental animals (rats and mice) to a high dose of a medicinal plant or plant product is necessary and valid in determining human hazards. Gamaniel (2000) suggested that the drug should be administered by the route intended for clinical use, and in addition one of the routes used should guarantee systemic absorption. Mortality is observed for three days (24–72 h) after parental administration and for 7 days after oral administration.

Medicinal plants have found a wide range of application in pharmacy, medicine, and traditional medicine. *Khaya grandifoliola*, tested here, has proved to be quite safe with a therapeutic dose of 12.4 mg/kg and an LD_{50} of 5.5 g/kg (i.e., >400-times the therapeutic dose).

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