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Anti-diarrhoeal and Toxicological Studies of Leaf Extracts of *Khaya senegalensis*

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ABSTRACT

The anti-diarrhoeal, phytochemical and toxicological properties of leaf extracts of *Khaya senegalensis* were evaluated. Anti-diarrhoeal activities of the extracts were evaluated on castor oil-induced diarrhea in rats and on small intestinal transit. Phytochemical and toxicological studies were carried out using standard methods. The aqueous and methanolic extracts at doses of 100-300 mg kg^{-1} significantly ($p<0.05$) reduced the onset of diarrhea and also showed a significant ($p<0.05$) reduction in gastrointestinal motility on charcoal meal test in rats. The aqueous extract appeared to be more effective than the methanolic extract. Phytochemicals detected in the extracts are saponins, flavonoids, tannins, steroids, alkaloids, glycosides and volatile oils. The LD_{50} of the aqueous extract was greater than 3000 mg kg^{-1} per os in rats. Sub-chronic administration of the aqueous extract at 600-3000 mg kg^{-1} for 28 days resulted in non significant ($p>0.05$) changes of the renal and liver indices and no histopathological changes of the organs were observed. The results of the study support the traditional use of the plant for diarhoeal remedies and also indicate that the plant has no toxic effect at all the concentrations employed.

Key words: *Khaya senegalensis*, anti-diarrhoeal activity, castor oil, toxicity studies, phytochemicals

INTRODUCTION

Diarrhoea is one of the major causes of child morbidity and mortality in the developing countries (Watcho *et al*., 2005). Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 years. WHO estimates that in 1998, about 7.1 million deaths occurred due to diarrhoea (Park, 2000). As a result of this, the World Health Organization (WHO) has set up a diarrhoeal disease control programme (CDD) which includes traditional medicine practices (Anonymous, 1979). Some plants have been evaluated for their anti-diarrhoeal properties (Mukherjee *et al*., 1998; Galves *et al*., 1993; Shoba and Thomas, 2001). Many medicinal plants used traditionally for anti-diarrhoeal treatment have been in use by man without the actual knowledge of their toxicological and pharmacological potential(s). *Khaya senegalensis* is an example of such a plant which has medicinal properties.

The plant *K. senegalensis* is a savanna tree, easily recognized by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules (Kubmarawa *et al*., 2008). The stem-back and leaves of *K. senegalensis* were used for the cure of mucous diarrhoea, syphilis, pyrexia and malarial fever (Olayinka *et al*., 1992). The aqueous extract of *K. senegalensis* was used

as a remedy for malaria (Gill, 1992). The plant was also reported to possess an *in vitro* trypanosomal activity (Wurochekke and Nok, 2004). To our knowledge there is no previous report on anti-diarrhoeal and toxicological properties of leaves extracts of *Khaya senegalensis*. Thus, the aim of this study is to evaluate the anti-diarrhoeal and toxicity/risk profiles of *Khaya Senegalensis*.

MATERIALS AND METHODS

Animals: Wistar strains albino rats of both sexes were obtained from the faculty of Pharmaceutical Sciences Ahmadu Bello University Zaria (ABU). The rats were fed with pellet feeds (Vital feeds Bukuru, Jos) and water *ad libitum*. They were kept in wire mesh cages at room temperature for two weeks to acclimatize. The study was done between December 2009 and February 2010. Animal treatment and handling were done according to the ethical guideline as reported by Zimmerman (1983) and in accordance with US guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 18-23).

Collection of plant material: The leaves of *K. senegalensis* were obtained in December (2009) from Gummi village, Gummi Local Government, Zamfara State, Nigeria. The plant was identified at the herbarium, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria and the voucher specimen (No. 025) was retained in the Herbarium.

Preparation of plant material: The leaves collected were open-air-dried under shade, pulverized into coarse powder (with a wooden pestle and mortar) and sieved with a 1 mm^2 and stored in a plastic container until required.

Preparation of the aqueous extract: The dried powdered leaves (550 g) was macerated with 5 L of distilled water at room temperature for 48 h and filtered through muslin cloth and then Whatman No. 1 filter paper. The filtrate was concentrated to dryness in an oven at 50°C and the percentage yield was 11.07% (w/w). The extract was stored in sealed plastic containers until needed. The dried powdered residue of the extract was further reconstituted in distilled water at different concentrations for oral administration to albino rats.

Soxhlet extraction: The powdered material (70 g) was exhaustively extracted with methanol (500 mL) using soxhlet extractor. The extract was concentrated and evaporated to dryness in an oven at 45°C.

Drugs and chemicals: Loperamide and Indomathacin (standard reference anti-diarrhoeal drugs), Castor-oil (laxative) charcoal meal (activated carbon) all of standard grades were used.

Phytochemical analysis: This was done using the procedures of Harborne (1973), Trease and Evans (1978) and El-Olemyl *et al*. (1994).

Acute toxicity studies (determination of LD_{50}): Aqueous extract of *K. senegalensis* (3000 mg kg^{-1} body weight) was administered (1 mL) to 5 groups (one rat per group) of rats one after the other at a grace observation period of 48 h in a single oral dose using a feeding syringe. The control group received distilled water. Observations of toxic symptoms like increased motor activity, arching and rolling, writhing, depression, salivation, loss of hair and death if any were

made and recorded systematically at 1, 2, 4 and 6 h after administration. The number of survivors was noted after 48 h for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated by using the limit test dose of up and down procedure of OECD (2001).

Castor oil-induced diarrhoea: Forty-eight rats were allowed to fast for 18 h and were divided into 8 groups of 6 animals each. The animals in group 1 served as negative control and received distilled water orally. Group 2 was positive control and received 5 mg kg^{-1} (b.wt.) of the standard drug Loperamide. Groups 3, 4 and 5 received 100, 200 and 300 mg kg^{-1}, respectively of the aqueous extract of the plant while groups 6, 7 and 8 received 100, 200 and 300 mg kg^{-1} (b.wt.), respectively of the methanolic extract of the plant. After 1hr of the drug pre-treatment, castor oil (12 mL kg^{-1}) was administered orally to all the groups. The animals were then placed in observation cages singly over clean filter paper and observed for defecation up to 6 h. The total number of watery stools was counted (Watcho *et al.*, 2005).

Castor oil-induced fluid accumulation: Forty-eight rats were allowed to fast for 24 h and were divided into 8 groups of 6 animals each. The animals in group 1 served as negative control and received distilled water orally. Group 2 served as a positive control and received 3 mg kg^{-1} (b.wt.) of the standard drug Indomethacin. Groups 3, 4 and 5 received 100, 200 and 300 mg kg^{-1} of the aqueous extract of the plant. Groups 6, 7 and 8 received 100, 200 and 300 mg kg^{-1} (b.wt.) of the methanolic extract of the plant. After 1 h of the drug pre-treatment, castor oil (12 mL kg^{-1}) was administered orally to all the groups. The animals were then sacrificed 1hr later. Their intestines were removed and the volume of intestinal contents were measured (Robert *et al.*, 1976).

Gastrointestinal motility (small intestinal transit time): Fourty-eight animals were fasted for 24 h and then placed in 8 groups of 6 animals each. Group 1 was administered with 10 mL kg^{-1} of normal saline orally. Group 2 received the standard drug Loperamide 5 mg kg^{-1} (b.wt.). Groups 3, 4 and 5 received 100, 200 and 300 mg kg^{-1} (b.wt.) of the aqueous extract of *K. senegalensis,* groups 6, 7 and 8 received 100, 200 and 300 mg kg^{-1} (b.wt.) of the methanolic extract. After 30 min of the extract administration, each animal was orally administered with 1 mL of charcoal meal (10% activated charcoal in distilled water). The rats were then sacrificed thirty minutes later and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of the total length of the intestine (Mujumdar, 1998; Mascolo *et al.*, 1994).

Body weight: The body weights of all the animals before the administration of the extract and weekly were recorded.

Sub-acute toxicity: Thirty-six rats were divided into the following groups; Group A (n = 6) was control group and received distilled water. Groups B, C, D, E and F (6 animals each) were orally administered (1 mL of 600, 1200, 1800, 2400 and 3000 mg kg^{-1} body weight) with aqueous leaves extract of *K. senegalensis* once daily for 28 days, respectively.

Biochemical parameters: Animals were sacrificed on the 29th day and blood samples were collected, allowed to clot and centrifuged to obtain sera. Serum Alanine Aminotransferase (ALT)

and Aspartate Aminotransferase (AST) were determined by using Randox assay kit (Reitman and Frankel 1957). Alkaline phosphatase activity was estimated by the randox kit (Colorimetric) of Rec GSCC (1972). Total Protein was determined by the biuret method as described by Gornall *et al.* (1949). Total Bilirubin (TBL) and Conjugated Bilirubin (CBL) were analyzed (Randox kits) using the methods of Jendrassik and Grof (1938) and Sherlock (1951). Albumin (Bromocresol green) and Urea (Diacetylmonoxime) were done by the methods of Cheesbrough (1991) and Wybenga *et al.* (1971), respectively. Uric acid was estimated by the method of Collins and Diehl (1959) and Morin and Prox (1973). Electrolytes and Creatinine (Colorimetric with deproteinization) were estimated by the methods of Uriyo and Singh (1974) and Henry (1974).

Histopathological assessment: Histopathological examinations were carried out on the liver and kidney of the rats. They were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin. Sections (4-6 μM thick) were prepared and then stained with hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light microscope at high power magnifications (×200 objective) (Wasfi *et al.*, 1994; Rao *et al.*, 2006).

Statistical analysis: Results are expressed as Mean±Standard error of mean. The data collected were subjected to one way Analysis of Variance (ANOVA), using Graph Pad Instat, Turkey Krammer compare all columns and significance was considered at $p<0.05$ and $p<0.01$.

RESULTS

Percentage yield is 11.07 and 45.6% for the aqueous and methanolic extracts of *K. senegalensis* (Table not shown).

Phytochemical screening: Phytochemical analysis (Table 1) of both extracts revealed the presence of flavonoids, tannins, steroids, alkaloids, glycosides, saponin glycosides, cardiac glycosides and volatile oils, with exception of saponin in the methanolic extracts and balsams and phlobatannin in methanolic extracts. Anthraquinone was not detected in the extracts.

Castor oil-induced diarrhoea in rats: Castor oil induced diarrhoea in some of the rats after 4 h of administration (Table 2). Pretreatment of rats with the aqueous and methanolic extract

Table 1: Phytochemical constituents of aqueous and methanolic leaf extracts of *Khaya senegalensis*

Phytochemicals	Methanolic extract	Aqueous extract
Steroids	+++	++
Cardiac glycosides	+++	+
Saponin glycosides	+++	+++
Glycosides	+++	++
Anthraquinones	-	-
Flavonoids	+++	++
Tannins	+++	+++
Saponins	-	++
Alkaloids	+++	+++
Balsams	++	+
Phlobatanin	+	-
Volatile oil	++	++

+: Presence (trace amount), ++: Presence (medium), +++: Presence (high) -: Absence

Table 2: Effect of aqueous and methanolic leaf extracts of *Khaya senegalensis* on castor oil-induced diarrhea in rats

Treatment	Dose (mg kg^{-1})	Diarrhoeal droppings	% inhibition
Distilled water		4.50±1.65	-
Loperamide	5	0.00±0.00**	100.00
Aqueous extract	100	2.17±1.01	51.80
Aqueous extract	200	0.50±0.22*	88.90
Aqueous extract	300	0.00±0.00**	100.00
Methanol extract	100	3.00±1.01	33.30
Methanol extract	200	2.00±0.58	55.60
Methanol extract	300	0.00±0.00**	100.00

Values are Means±SEM. **=significantly different at P<0.01, * significantly different at P<0.05, by using analysis of variance, Turkey Kramer Multiple Comparisons, Graph Pad Instat Software (San Diago, USA)

Table 3: Effect of aqueous and methanolic leaf extracts of *Khaya senegalensis* on intestinal fluid accumulation in rats

Treatment	Dose (mg kg^{-1})	Fluid volume (mL)	% inhibition
Distilled water		2.45±0.21	-
Indomethacin	5	1.04±0.12	57.0
Aqueous extract	100	1.84±0.18	24.9
Aqueous extract	200	1.86±0.12	24.1
Aqueous extract	300	1.33±0.10*	45.7
Methanol extract	100	1.83±0.11	25.3
Methanol extract	200	1.94±0.12	20.8
Methanol extract	300	2.23±0.22	10.0

Values are Mean±SEM. **Significantly different at p<0.01, *significantly different at p<0.05 by using analysis of varience, Turkey Kramer multiple comprisons, graph pad instat software (San Digo USA)

(100, 200, 300 mg kg^{-1}) caused a dose-dependent and significant ($p<0.05$) reduction in the number of diarrhoeal droppings. The aqueous extract had a more significant effect than the methanolic extract (Table 2). The highest dose of the extract 300 mg kg^{-1} had the highest effect and was the same as the standard drug loperamide.

Castor oil-induced fluid accumulation in rats: The results of the effect of the aqueous and methanolic leaves extracts of *K. senegalensis* on the intestinal fluid accumulation are presented on Table 3. Oral administration of castor oil to the rats in the control group produced intestinal fluid volume of 2.45±0.21 mL. The aqueous extract of *K. senegalensis* significantly ($p<0.05$) inhibited the fluid accumulation in castor oil treated rats only at 300 mg kg^{-1} with fluid volume of 1.33±0.10. While the methanolic extracts and the rest of the aqueus extract had effects that were not very significant ($p>0.05$). However, the aqueous extract was more effective.

Gastrointestinal transit: The aqueous and methanolic extract of *K. senegalensis* (100, 200 and 300 mg kg^{-1} p.o.) inhibited the intestinal propulsion in charcoal meal-treated rats dose dependently. The aqueous extract at 200 and 300 mg kg^{-1} has an inhibition of 35% similar to loperamide and was more effective than the methanolic extract. The inhibition of the intestinal propulsion of the charcoal meal by the methanolic extract was not significantly different ($p>0.05$) when compared to the control group (Table 4).

Acute toxicity study and behavioural effects: Oral administration of aqueous leaves extract of *K. senegalensis* at a single dose of 3000 mg kg^{-1} body weight did not produce significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, loss of hair and

Table 4: Effect of aqueous and methanolic extracts of *Khaya senegalensis* on normal intestinal transit in rats

Treatment	Dose mg kg^{-1}	IL (cm)	CML (cm)	% IT	% inhibition
Distilled water		98.00±3.810	89.20±2.00	91.38±2.84	-
Loperamide	5	104.00±4.01	60.40±4.29	58.65±5.37**	36
Aqueous extract	100	105.20±2.65	80.60±3.27	76.57±2.08	16
Aqueous extract	200	100.20±3.56	71.86±5.09	71.72±3.06*	22
Aqueous extract	300	110.20±5.21	65.10±6.70	58.73±4.14**	35
Methanol extract	100	102.00±4.43	86.20±8.59	77.98±5.85	14
Methanol extract	200	109.60±3.49	84.80±7.20	77.02±4.76	15
Methanol extract	300	95.37±1.630	79.40±2.21	83.34±3.84	08

Values are Mean±SEM. **: Significantly different at p<0.01, *Signifieatat p<0.05 by using analysis of variance, Turkey Kramer Multiple Comparisons, Graph Pad Instat Software (San Diago, U.S.A.). CML: Charcoal meal length, IL: Intestinal length, IT: Intestinal transit

Table 5: Liver function indices of rats administered with aqueous leaf extracts of *Khaya senegalensis*

Dose (mg kg^{-1})	ALT (μ L^{-1})	AST(μ L^{-1})	ALP(μ L^{-1})	ALB (g dL^{-1})	TP (g L^{-1})	TB (Mg dL^{-1})	CB (Mg dL^{-1})
Control	11.8±0.49	14.2±1.53	88.0±5.83	3.15±0.07	6.80±0.16	0.39±0.04	0.16±0.01
600	10.4±0.98	14.2±1.53	84.0±4.00	3.56±0.06	5.81±0.12	0.41±0.04	0.17±0.03
1200	10.8±0.80	17.2±0.73	82.0±2.00	3.73±0.17*	5.78±0.35	0.45±0.02	0.15±0.01
1800	11.8±1.36	16.6±0.60	88.0±3.83	3.30±0.15	4.94±0.44	0.43±0.07	0.17±0.02
2400	9.2±0.490	13.6±0.60	82.0±2.00	3.55±0.63	5.88±0.14	0.58±0.08	0.24±0.03
3000	10.0±0.89	13.0±1.34	84.0±2.45	3.23±0.47	5.74±0.10	0.39±0.04	0.26±0.02*

Values are Mean±SEM. *: Significantly different (p<0.05), using analysis of variance (ANOVA), Turkey Kramer Multiple Comparisons, Graph Pad Instat Software (San Diago, USA). ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline phosphatase, ALB: Albumin, TP: Total protein, TB: Total Bilirubin, CB: Conjugated bilirubin, (n = 6)

Table 6: Renal function indices of rats administered with aqueous leaves extracts of *Khaya senegalensis*

Dose (mg kg^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	Na^+ (mmol^{-1})	K^+ (mmol^{-1})	HCO_3^- (mmol L^{-1})
Control	35.02±5.02	0.61±0.02	4.63±1.13	130.4±2.69	5.12±0.24	24.2±1.07
600	38.04±3.39	0.93±0.17*	1.88±0.19	126.0±0.00	5.02±0.19	24.8±1.07
1200	37.74±3.06	0.66±0.02	2.88±0.42	133.8±3.23	5.06±0.18	21.6±0.40
1800	44.67±8.75	0.62±0.02	2.75±0.51	137.2±2.80	5.10±0.10	25.6±1.50
2400	33.6±2.980	0.60±0.03	2.50±0.56	136.6±2.71	5.2±0.120	27.0±1.52
3000	30.79±3.26	0.63±0.03	3.25±0.64	128.0±1.38	5.10±0.10	27.2±1.39

Values are Mean±SEM, (n = 6), Significant from control group *p<0.05; **p<0.01 by using Analysis of Variance (ANOVA),Turkey Kramer Multiple Comparisons, Graph Pad Instat Software Software (San Diago, USA) Software (San Diago, USA) Software (San Diago, USA)

gastrointestinal effects on the test animals. These effects were observed during the experimental period of fourty-eight hours (48 h), the only observable sign or symptom of discomfort in the animals was the scratching of the cages as if the animal wanted to escape. The test animals only discontinued feeding temporarily for about 5 min after the administration of the extract but continued eating soon after and no deaths were recorded. These results showed that the medium lethal dose (LD_{50}) is greater than 3000 mg kg^{-1}.

Sub-chronic toxicity (hepatorenal functions): There was no significant differences (p>0.05) in most of the liver function indices observed. However, there was a significant increase (p<0.05) in serum albumin of the group administered with 1200 mg kg^{-1} and also a significant increase of conjugated bilirubin in animals administered with 30000 mg kg^{-1} (Table 5). All the kidney function indices were statistically non significant (p>0.05) (Table 6) with exception of creatinine level at 600 mg kg^{-1}.

Histopathological studies: There was no histopathological changes observed in the kidney and the liver of the animals administered with all doses of the aqueous leaves extracts of the plant.

DISCUSSION

From the results, the extracts have significantly ($p<0.05$) reduced the number of diarrhoeal droppings induced by castor oil. Induction of diarrhea by castor oil is attributed to its active ingredient ricinoleic acid (Gagniella *et al*., 1975) which causes irritation on gastric mucosa, inducing changes in mucosa fluid and electrolytes transport (Ammon *et al*., 1974). Ricinoleic acid also stimulates the production of several mediator substances that include prostaglandins, nitric oxide and platelet activating factor, cAMP and tachykinins (Izzo *et al*., 1999). Since the extract was capable of inhibiting the castor oil induced diarrhea, it is therefore probable that the antidiarrhoeal action exerted by the extract may be attributed to the inhibition of prostaglandin biosynthesis or release. The extract did not significantly inhibit the castor oil-induced intestinal fluid accumulation and this suggests that its antidiarrhoeal activity may not be related to anti-secretory action. Thus, the extracts of *K. senegalensis* have significantly reduced intestinal transit time. The decrease in intestinal motility will allow the intestinal content more time to be exposed to the absorptive surface of the intestinal tract (Friedman and Isselbacher, 1998).

The aqueous extract was more effective than the methanolic extract in all the models and may be due to the presence of saponins and other phytocompounds in the aqueous extracts. Flavonoids in plants have been demonstrated to inhibit contraction caused by spasmogenes (Macauder, 1986), inhibit intestinal secretion and small intestinal transit (Viswanathan *et al*., 1984; Di Carlo *et al*., 1993). The phytochemical diversity of plant specie is indicative of its high therapeutic potentials. This is because these compounds form the basis of the pharmacologic effects of such plants (Haidet, 2003; Jigam *et al*., 2004). Previous reports have demonstrated the antidiarrhoeal activity of tannins (Mukherjee *et al*., 1998), flavonoids (Galves *et al*., 1993), alkaloids (Shoba and Thomas, 2001), saponins and sterols (Otshudi *et al*., 2000). Thus, the presence of these compounds in the leaves extracts of *K. senegalensis* may be responsible for the antidiarrhoeal activity of the plant. This is in agreement with the findings of Kubmarawa *et al*. (2008) and Ojo *et al*. (2006) who also reported same phytochemicals in this plant.

The acute toxicity studies did not show any behavioural changes or signs of toxicity like excitement, arching and rolling, writhing, depression, salivation, loss of hair, coma and death. The LD_{50} obtained was greater than 3000 mg kg^{-1}. The administration of the extract did not result in any significant reduction in weight gain or weight loss. In the sub-chronic toxicity studies, almost all the indices of the liver and kidney functions did not vary significantly when compared to those in the control animals. Assay of enzyme activities in tissues and body fluids are an important aid in disease investigation and diagnosis (Malomo, 2000). Alkaline phosphatase is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). Total bilirubin and albumin are 'markers' of liver excretory function (Cheesbrough, 1991) and synthetic function of the liver (Harold *et al*., 1980; Corless and Middleton, 1983; Cheesbrough, 1991). ALT and AST are liver specific enzyme 'markers' of necrotic injury and cholestasis (Spech and Liehr, 1983; Lott and Wolf, 1986). From the results, most of the liver and renal indices were not significantly ($p>0.05$) affected. However, Abubakar *et al*. (2009) has reported hepato toxicity of sub-chronic administration of aqueous extract of stem bark of *K. senegalensis*. But in this study, the leaves of the plant have shown no signs of toxicity at doses of 600 to 3000 mg kg^{-1}. The aqueous extract of *K. senegalensis* has also been reported to have hepatoprotective activity (Ojo *et al*., 2006). The

histopathological results obtained in this research did not show any signs of lesions or pathological changes in the liver and the kidney of rats. This has justified the results of the biochemical indices of the kidney and liver function.

In the present study, the aqueous and methanolic extracts of *K. senegalensis* have anti-diarrhoeal activity and the aqueous extract of the plant was more effective than methanolic extract. The toxicity studies did not reveal any apparently harmful or deleterious effect of leaves of *K. senegalensis*. Structural elucidation of the active agent(s) and mechanism of action are recommended.

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