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PHYTOCHEMICAL AND TOXICOLOGICAL ASSESSMENT OF AN AQUEOUS STEM BARK EXTRACT OF *PARKIA BIGLOBOSA* JACQ USED IN AN ANTIHEMORRHOIDAL FORMULATION

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ABSTRACT

Parkia biglobosa is a plant widely used in traditional medicine for the treatment of various diseases, including hemorrhoids. Several parts of the plant, including the bark, are used for this purpose. This study aimed to perform phytochemical characterization and evaluate the acute and subacute toxicity and mutagenicity of the aqueous stem bark extract of *Parkia biglobosa* intended for an anti-haemorrhoidal phytomedicine development. Phytochemical screening was carried out according to the methods described by Wagner and Balt. Acute toxicity was assessed at 2000 mg/kg body weight (bw) in female Wistar rats, subacute toxicity over 28 days in male and female Wistar rats at doses of 250, 500 and 1000 mg/kg bw, and mutagenicity over 7 days in NMRI mice at doses of 500, 1000 and 2000 mg/kg bw, in accordance with the adapted guidelines of the Organization for Economic Co-operation and Development. The chemical groups detected in the extract were flavonoids, sterols, triterpenes, saponosides, and tannins. In the acute test, no toxic effects were observed; the LD50 was

estimated to exceed 2000 mg/kg bw. Also, no major repeated-dose toxicity or increase in micronucleated erythrocytes was noted in the subacute and mutagenicity assessment. As the extract evaluated did not cause any proven mutagenic effects in NMRI mice or any major acute and subacute toxicity effects in Wistar rats, it was considered relatively non-toxic in treated animals at the doses evaluated.

KEYWORDS: *Parkia biglobosa*, phytochemistry, lethal dose 50, oral toxicity, mutagenicity, herbal medicine.

1. INTRODUCTION

Globally, the use of traditional medicine (TM), particularly herbal medicines, has grown over the last two decades, and many people now use it to treat a variety of illnesses.[1] In some countries, traditional or non-conventional medicine is referred to as complementary medicine (CM).[2] Thus, throughout the world, traditional medicine is either the primary means of healthcare delivery or a complement to it.

Many populations in developing countries use it because official medical care is often based on commercial medicines, which are expensive, whereas a traditional medical consultation is much cheaper.[3]

This is why the World Health Organization (WHO), in its document "Strategy for Traditional Medicine 2014-2023", has set itself the goal of supporting countries that harness the contribution of traditional medicine to health and well-being, and promoting the safe and effective use of traditional medicine through regulation.[2]

Today, several studies aimed at better understanding plants, formalizing their medicinal uses, and promoting their benefits are being conducted worldwide, particularly in Africa.

Among the plants most commonly used by the Sahelian and Sudanese populations of West Africa is *Parkia biglobosa (P. biglobosa)*. It is a species of the Leguminosae family, subfamily Mimosoideae, and tribe Mimosaceae. It belongs to the *Parkia* genus. There are about thirty species distributed across three distinct centers of diversity in South America, Africa, and Asia.[4]

In addition to its nutritional and socio-economic value, this species is widely used by local populations to treat various illnesses. The bark, leaves, and roots are the parts of the plant

most commonly used for this purpose. The bark was the most widely used part, according to the large number of reported uses. The plant is mainly used to treat digestive disorders, wounds, high blood pressure, and infections.[5]

However, while many countries agree on the need for cohesive and comprehensive access to healthcare, it is essential to provide those seeking care with traditional medicine and pharmacopeia that are safe, respectful, cost-effective, and efficient.[6]

To promote *P. biglobosa* in the medical field, numerous studies have been conducted, particularly in pharmacology, phytochemistry, toxicology, traditional medicine, and ethnobotany. However, toxicological data are poorly documented and mainly concern acute toxicity studies. Furthermore, given the differences that may exist in the composition of plant extracts depending on the harvest site[7] and the extraction method,[8] certain toxicity data on the plant, albeit limited, must be included to meet this requirement for diversity related to the geography of the harvest site Hence, the need to focus research on this plant, widely used in traditional African medicine, on its toxicological aspects to ensure its safe use.

This study aimed to characterize the phytochemistry and evaluate the acute and subacute toxicity and mutagenicity of the aqueous stem bark extract of *Parkia biglobosa* Jacq intended for the formulation of an anti-hemorrhoidal phytomedicine prototype.

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Raw powder

The stem bark of *Parkia biglobosa* was harvested in January 2024 in Baré, a village located 25 km from Bobo-Dioulasso in Burkina Faso. A specimen was identified and registered under number 8757 with the geographical coordinates (X = 378575; Y = 1223464) in the National Herbarium of BURKINA FASO (HNBU) at the National Center for Scientific Research and Technology (CNRST). The bark was dried in a ventilated room, away from sunlight and at room temperature. The dry bark was then ground using a Gladiator hammer mill and stored in airtight bags. Powdered bark from the trunk of *Parkia biglobosa* was provided by the Institute for Health Sciences Research (IRSS) in Ouagadougou, Burkina Faso.

2.1.2. Aqueous extract of stem bark of *Parkia biglobosa* powder preparation

Aqueous extract of powder from the stem bark of *P. biglobosa* was prepared following the ratio of 1 g of powder to 25 mL of distilled water. For each test, 40 g of powder was placed in a flask, and 1 L of distilled water was added.

The flask containing the mixture, coupled with a reflux condenser, was placed on a hot plate set at 50°C. Once boiling commenced, the flask was kept on the hot plate for 20 minutes. The resulting decoction was then cooled and filtered through a fine-mesh nylon cloth. The filtrate was then centrifuged at 2000 rpm for 10 minutes and filtered again using a coffee filter. This filtrate was frozen and freeze-dried, and the freeze-dried product was then used for the activities.

2.2. Animal material

The animal material consisted of male and female NMRI mice for mutagenicity, female Wistar rats for acute toxicity assessment, and male and female Wistar rats for subacute toxicity assessment. The animals were provided by the International Center for Research and Development in Subhumid Livestock Farming (CIRDES), Burkina Faso. They were raised at an ambient temperature of 22°C (±3) and a humidity of 60% (±5) in the animal facility of the Phytomedicines and Medicines Research and Development Laboratory of the Health Sciences Research Institute. They were fed protein-enriched pellets, had access to running tap water, and were subjected to 12 hours of light and 12 hours of darkness.

2.3. Phytochemical screening

Phytochemical screening was performed on CCM silica gel 60 F254 chromatographic plates according to methods described in the literature.[9,10] The aim was to identify major chemical groups, including coumarins, flavonoids, alkaloids, sterols, triterpenes, saponosides, tannins, and anthracenosides, using thin-layer chromatography (TLC).

The freeze-dried aqueous extract was dissolved in methanol, and deposits were made on TLC plates for each chemical group. The plates were then migrated in the tank containing the appropriate migration solvent for each chemical group. After migration, the plates were dried and, as necessary, sprayed with the developer. The chromatographic profiles were observed in visible light and under a UV/Vis lamp (254 and 365 nm) before and after development.

2.4. Acute toxicity

The method used for the acute toxicity test was based on that described in the Organisation for Economic Cooperation and Development (OECD) Guideline 423[11], adapted to the Toxicology and Ecotoxicology Laboratory of the Health Sciences Research Institute, Ouagadougou (Burkina Faso).

Female Wistar rats with an average weight of 146 ± 3,07 g were used to assess acute toxicity. The animals were fasted for 4 hours prior to administration of the test substance. The individual weight of each animal was determined shortly before administration.

Two groups of three female rats, each individually marked, were formed. The animals in each group were placed in a cage and treated as follows:

- Group 1, the control group, received tap water;
- Group 2, the test group, received the aqueous extract of *P. biglobosa* bark at a dose of 2000 mg/kg body weight.

The extract was administered orally in a single dose using an oesophageal probe. The volume administered was 2 mL/100 g body weight.

The animals were observed individually once during the first 30 minutes and regularly during the first 24 hours after treatment. Particular attention was paid to monitoring during the first 4 hours and daily for 14 days after administration of the test extract. The test was repeated under the same conditions to confirm the results of the first test.

2.5. Subacute toxicity

It was based on the method described in Organization for Economic Cooperation and Development (OECD) Guideline 407,[12] adapted to the realities of the Toxicology and Ecotoxicology Laboratory.

Healthy adult male and female Wistar rats with an average weight of 183.9 ± 10,60 g were used. The females were nulliparous. Four groups of five (5) female rats and four groups of five (5) male rats were used, including three test groups and one control group. The control groups were fed tap water via gastric tube for 28 days, while groups L1, L2, and L3 of each sex received 250, 500, and 1000 mg/kg body weight of the test extract, respectively.

The animals were individually identified and placed in cages five days before the start of the study.

The aqueous stem bark extract of *Parkia biglobosa* was administered to the rats daily by gavage at a single dose using a gastric tube, seven days a week, for 28 days. The volume administered was 2 mL/100 g body weight.

During the test, the animals underwent a general clinical examination at least once a day, at the same time. Water and food consumption and weight changes were monitored daily throughout the study.

On the twenty-ninth day, after fasting, all rats were anesthetized, and their blood was collected by cardiac puncture and collected in dry tubes. The blood samples were centrifuged at 3000 rpm for 10 minutes, and the sera were collected for biochemical analysis.

After a general autopsy, vital organs such as the liver, lungs, heart, spleen, and kidneys were removed, and their relative weights (%) were assessed using the following formula:

$$Relative\ Organ\ Weight\ (ROW\ (\%)) = \frac{Weight\ of\ the\ organ}{Weight\ of\ the\ animal\ on\ the\ day\ of\ slaughter} \times 100$$

2.6. Mutagenicity

The study of mutagenicity was based on Guideline 474 of the Organization for Economic Cooperation and Development (OECD)[13], adapted to the realities of the Toxicology and Ecotoxicology Laboratory.

Young adult NMRI mice were used and randomly divided into control and treatment groups. Each mouse was individually identified.

The test was performed on five groups of five mice per sex, including three test groups and two control groups (one positive control and one negative control).

The different groups were homogeneous, and the females were nulliparous and non-pregnant. Potassium bromate ($KBrO_3$) was used as a positive control. It was dissolved in a 0.9% NaCl solution. The volumes administered were proportional to the animals' body weight and were 1 mL/100g.[14]

The negative controls received distilled water orally, and the positive control received 80 mg/kg body weight of potassium bromate ($KBrO_3$) intraperitoneally. The three test groups received orally 500, 1000, or 2000 mg/kg body weight of the test extract, respectively. All these substances were administered to the animals once daily for one week.[14]

At the end of the test period, peripheral blood was collected from the tail vein of each mouse 48 hours after the last administration. Blood smears were prepared on coded microscope slides from this blood sample. These smears were dried for 20 minutes, then fixed and stained with Giemsa. These slides were examined using an Olympus optical microscope at ×100 magnification under immersion oil.[14]

The frequency of micronucleated polychromatic erythrocytes (MPE) and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) were determined for each mouse by counting one thousand (1000) erythrocytes.[14]

2.7. Ethical Approval

This study was approved by the Animal Experimentation Ethics Committee at Joseph KI-ZERBO University in Burkina Faso, under approval code CE-UJKZ/2026-3.

2.8. Statistical analysis

Data analyses were performed using Prism GraphPad software version 8.4.3. Differences were considered statistically significant at the 5% level ($p < 0.05$).

3. RESULTS

3.1. Phytochemical screening

The major chemical groups searched for by thin-layer chromatography (TLC) were coumarins, flavonoids, sterols, triterpenes, saponosides, tannins, and anthracenosides. The results of the characterization of the different chemical groups searched for, and their interpretations, are presented in Table I and Figure 1

Table I: Phytochemical screening by thin-layer chromatography to identify chemical groups in aqueous stem bark extract of *Parkia biglobosa* Jacq.

Chemical groups	Reagents	Results
Flavonoids	Neu's reagent	+
Alkaloids	*Dragendroff's* reagent	-
Tannins	2% alcoholic solution of iron chloride ($FeCl_3$)	+
Coumarins	5% alcoholic solution of potassium hydroxide	-

Triterpenes and sterols	*Liebermann- Burchard* reaction	+
Saponosides	Sulphuric anisaldehyde	+
Anthracenosides	5% potassium hydroxide alcoholic solution	-

+ = detected; - = not detected

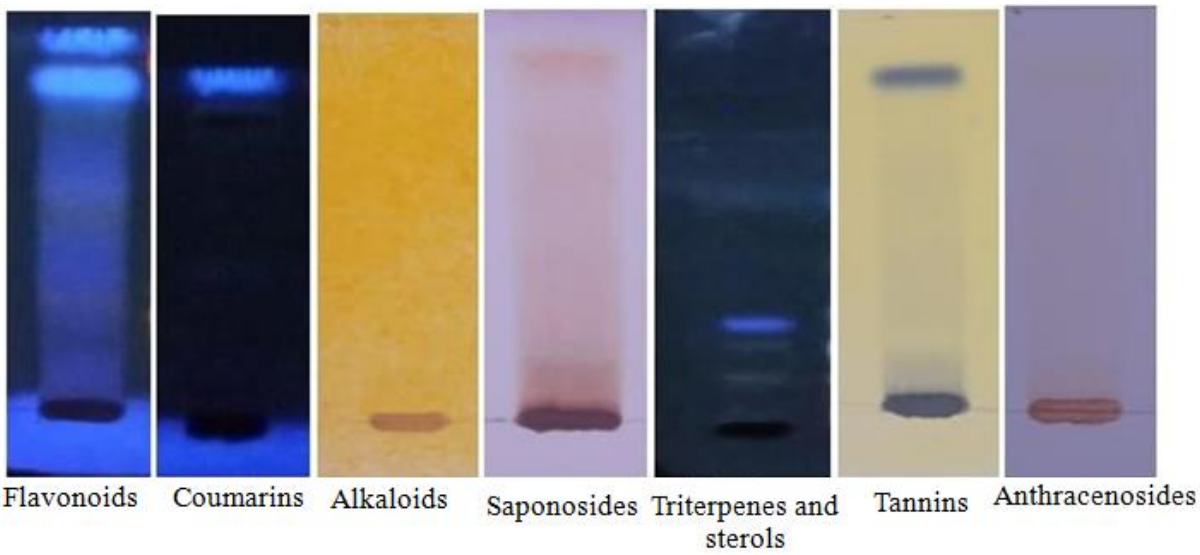


Figure 1: Thin-layer chromatography (TLC) for the detection of chemical groups in the aqueous stem bark extract of *Parkia biglobosa* Jacq.

3.2. Acute toxicity

Administering a single dose of 2000 mg/kg body weight of the extract to Wistar rats did not cause mortality in either test. There was no significant difference ($p > 0.05$) in body weight between the control rats and those that received the extract. Monitoring the animals over fourteen days revealed no major behavioral differences between the test and control groups. As a result, the lethal dose 50 (LD_{50}) of the extract was estimated to be greater than 2000 mg/kg body weight. The results are shown in Table II and Figure 2.

Table II: Evaluation of the acute oral toxicity of the aqueous stem bark extract of *Parkia biglobosa* in female Wistar rats at a dose of 2000 mg/kg body weight.

Doses	**Mortality of rats at 2000 mg/kg b.w. Doses**	
	First test (n=3)	Second test (n=3)
Control	0/3	0/3
2000 mg/kg	0/3	0/3

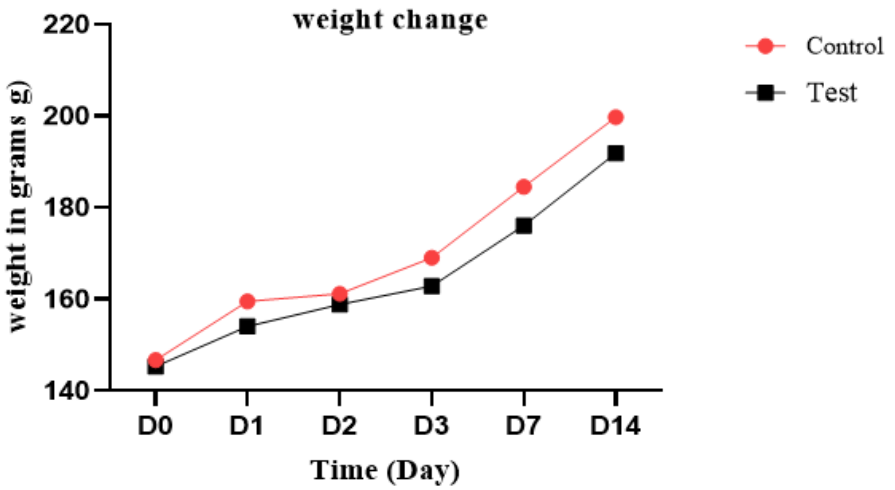


Figure 2: Body weight changes in rats treated with an aqueous stem bark extract of *Parkia biglobosa* at a dose of 2000 mg/kg body weight for the assessment of acute oral toxicity.

3.3. Subacute toxicity

3.3.1. Evaluation of water consumption

During the study period, water consumption in rats treated with the extract and in control rats remained around the average, with no significant increase or decrease observed in either sex. No significant difference in water consumption was observed between the control and test groups for either sex or dose. The results are shown in Table III.

Table III: Evaluation of daily water consumption (mL/rat/day) in rats over twenty-eight days.

Time	Sex	Controls	250 mg/kg	500 mg/kg	1000 mg/kg
Week 1	M	59.86 ± 12.13	55.14 ± 8.17	58.29 ± 6.37	55.00 ± 5.00
	F	61.86 ± 12.65	53.57 ± 8.02	61.71 ± 3.73	55.00 ± 2.89
Week 2	M	62.14 ± 9.51	59.86 ± 5.64	55.00 ± 4.08	59.57 ± 4.61
	F	47.43 ± 3.36	47.14 ± 5.67	55.00 ± 9.13	53.86 ± 4.56
Week 3	M	57.86 ± 11.50	58.00 ± 2.52	59.29 ± 7.32	58.57 ± 3.78
	F	45.71 ± 6.07	45.29 ± 4.72	50.00 ± 8.66	52.86 ± 2.67
Week 4	M	62.86 ± 6.99	56.43 ± 3.78	57.14 ± 13.18	52.86 ± 3.93
	F	44.86 ± 2.67	46.43 ± 4.76	48.86 ± 2.73	49.29 ± 3.45

Data presented as mean ± standard deviation, with n = 5

3.3.2. Evaluation of food consumption

The food consumption in the control and test groups remained stable during the first three weeks, followed by a slight decrease in the fourth week. Except for the group of male rats that received the 500 mg/kg dose and the group of female rats that received the 250 mg/kg

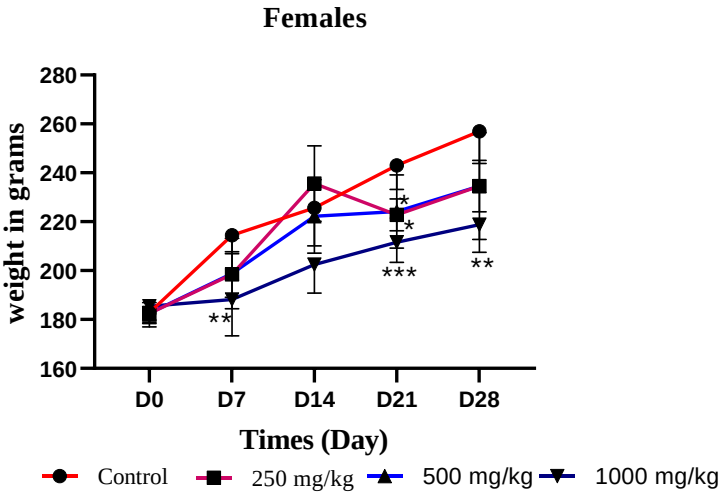
dose, for which an increase in food consumption was observed throughout the trial period. However, there was no significant difference (P > 0.05) in the change in daily food consumption per rat between the control and test groups in both sexes, regardless of the dose. The results are shown in Table IV.

Table IV: Evaluation of daily food consumption (g/rat/day) of rats over twenty-eight days.

Doses (mg/kg/b.w.)					
Time	**Sex**	**Control**	**250**	**500**	**1000**
Week 1	M	22.94	21.03	20.34	20.25
	F	21.06	18.37	19.23	16.86
Week 2	M	25.91	25.80	23.54	21.14
	F	22.91	19.17	20.91	17.37
Week 3	M	27.00	26.63	24.20	20.29
	F	20.49	19.37	21.91	18.66
Week 4	M	26.06	25.29	25.74	15.94
	F	20.43	19.66	18.31	17.63

3.3.3. Weight change

During the trial period, all rats gained weight. However, there was a significant difference (P < 0.05) in weight gain between the control group and the 250 and 500 mg/kg dose groups in females from the third week onwards, and a significant difference between the control group and the 1000 mg/kg dose group from the second week onwards in females and from the third week onwards in males, and throughout the remainder of the trial period. The results are shown in Figure 3.



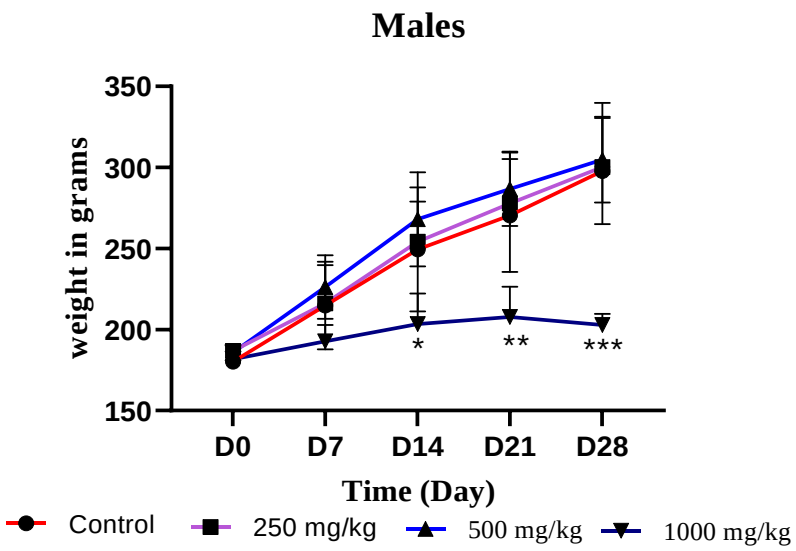


Figure 3: Weight change in male and female rats treated with aqueous stem bark extract of *Parkia biglobosa* compared with the control groups over the test period.

3.3.4. Relative organ weights

The relative weights of vital organs, including the lungs, liver, heart, spleen, and kidneys, were assessed. The analyses were performed using one-way ANOVA for each organ and sex. A significant difference ($p < 0.05$) in relative organ weight was observed for the spleen in both sexes at the 1000 mg/kg dose compared to each control group of the same sex. However, no significant differences in relative organ weight were observed for the other organs (liver, lungs, heart, and kidneys) between the extract-treated and control groups in both sexes. The data are shown in Table V.

Table V: Evaluation of the relative weight of vital organs in male and female rats treated with the aqueous stem bark extract of *Parkia biglobosa* compared with the control groups.

Doses (mg/kg/b.w.)	Sex	Liver	Lungs	Spleen	Heart	Kidneys
Control	M	3.17 ± 0.26	0.62 ± 0.10	0.37 ± 0.07	0.36 ± 0.02	0.66 ± 0.05
	F	3.55 ± 0.11	0.71 ± 0.08	0.47 ± 0.04	0.36 ± 0.06	0.66 ± 0.07
250	M	3.06 ± 0.23	0.66 ± 0.21	0.45 ± 0.04	0.32 ± 0.03	0.64 ± 0.01
	F	3.23 ± 0.44	0.55 ± 0.09	0.50 ± 0.10	0.33 ± 0.06	0.63 ± 0.08
500	M	2.95 ± 0.31	0.61 ± 0.12	0.41 ± 0.07	0.35 ± 0.02	0.61 ± 0.05
	F	3.34 ± 0.27	0.67 ± 0.11	0.46 ± 0.09	0.40 ± 0.06	0.68 ± 0.07
1000	M	2.81 ± 0.23	0.55 ± 0.07	0.30 ± 0.10*	0.35 ± 0.03	0.66 ± 0.04
	F	3.03 ± 0.30	0.69 ± 0.09	0.32 ± 0.06**	0.37 ± 0.07	0.73 ± 0.04

*Data presented as mean ± standard deviation, with n=5; *p<0.05, **p<0.01*

3.3.5. Evaluation of biochemical parameters

Biochemical parameters, including blood creatinine, cholesterol, calcium, sodium, chloride, phosphorus, potassium, and transaminase (AST and ALT) concentrations, were measured and compared between control and treated rats. The analyses were performed using one-way ANOVA for each element and sex. No significant differences ($p > 0.05$) in creatinine, total cholesterol, total protein, phosphate, chloride, sodium, potassium, and ALT concentrations were found between control rats and treated rats. However, significant differences ($p<0.05$) were observed in serum calcium concentration in male rats treated with a dose of 500 mg/kg, and serum AST concentration in male rats treated with a dose of 1000 mg/kg. The data are shown in Table VI.

Table VI: Evaluation of biochemical parameters in male and female rats treated for 28 days with aqueous stem bark extract of *Parkia biglobosa*.

		Doses (mg/kg/b.w.)			
Biochimicals elements	**Sex**	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Creatinine (µmol/L)	M	57.54 ± 3.62	62.26 ± 1.87	61.96 ± 4.48	62.36 ± 1.05
	F	69.36 ± 2.46	72.86 ± 5.40	74.70 ± 5.56	65.54 ± 3.53
Total cholesterol (mmol/L)	M	0.85 ± 0.27	0.75 ± 0.10	0.72 ± 0.13	0.69 ± 0.18
	F	1.00 ± 0.17	0.89 ± 0.11	0.99 ± 0.07	0.84 ± 0.14
Total protein (g/L)	M	63.48 ± 4.17	60.84 ± 3.30	55.79 ± 7.11	63.60 ± 4.46
	F	70.50 ± 5.05	72.96 ± 4.63	70.86 ± 3.75	68.32 ± 4.86
Ca^{2+} (mmol/L)	M	2.61 ± 0.29	2.60 ± 0.17	2.15 ± 0.26**	2.64 ± 0.07
	F	2.82 ± 0.06	2.75 ± 0.13	2.65 ± 0.16	2.84 ± 0.12
PO_4^{2-} (mmol/L)	M	2.95 ± 0.31	2.90 ± 0.21	2.50 ± 0.53	2.65 ± 0.64
	F	3.07 ± 0.20	2.81 ± 0.71	2.94 ± 0.19	3.66 ± 0.72
Cl^- (mmol/L)	M	99.80 ± 3.42	102.40 ± 6.54	105.20 ± 2.17	105.80 ± 2.95
	F	105.00 ± 1.58	108.80 ± 3.03	105.40 ± 6.27	109.20 ± 4.44
Na^+ (mmol/L)	M	140.60 ± 6.88	144.00 ± 7.45	135.40 ± 6.47	144.60 ± 4.93
	F	148.40 ± 3.65	148.00 ± 4.06	141.80 ± 7.92	146.60 ± 4.39
K^+ (mmol/L)	M	5.74 ± 1.23	5.18 ± 0.58	5.16 ± 1.11	4.49 ± 0.58
	F	4.37 ± 0.69	4.91 ± 1.56	4.05 ± 1.03	4.93 ± 0.78
AST (UI/L)	M	115.40 ± 6.39	117.40 ± 14.40	133.80 ± 17.71	92.40 ± 8.56*
	F	117.60 ± 12.50	120.40 ± 13.43	123.80 ± 34.65	105.80 ± 8.67
ALT (UI/L)	M	35.00 ± 7.78	44.00 ± 6.20	39.20 ± 7.85	34.80 ± 3.42
	F	34.40 ± 8.44	32.80 ± 7.29	31.60 ± 5.55	34.60 ± 3.65

*Data presented as mean ± standard deviation, with n=5; *p<0.05, **p<0.01, ***p<0.001*

3.4. Mutagenicity

Analysis of data on the number of polychromatic erythrocytes in the test batches and the various controls indicated that there was no significant difference ($p > 0.05$) between the negative controls and the test batches for both sexes. However, a highly significant difference

($p < 0.01$) was observed between batches treated with the extract and those treated with potassium bromate in both sexes.

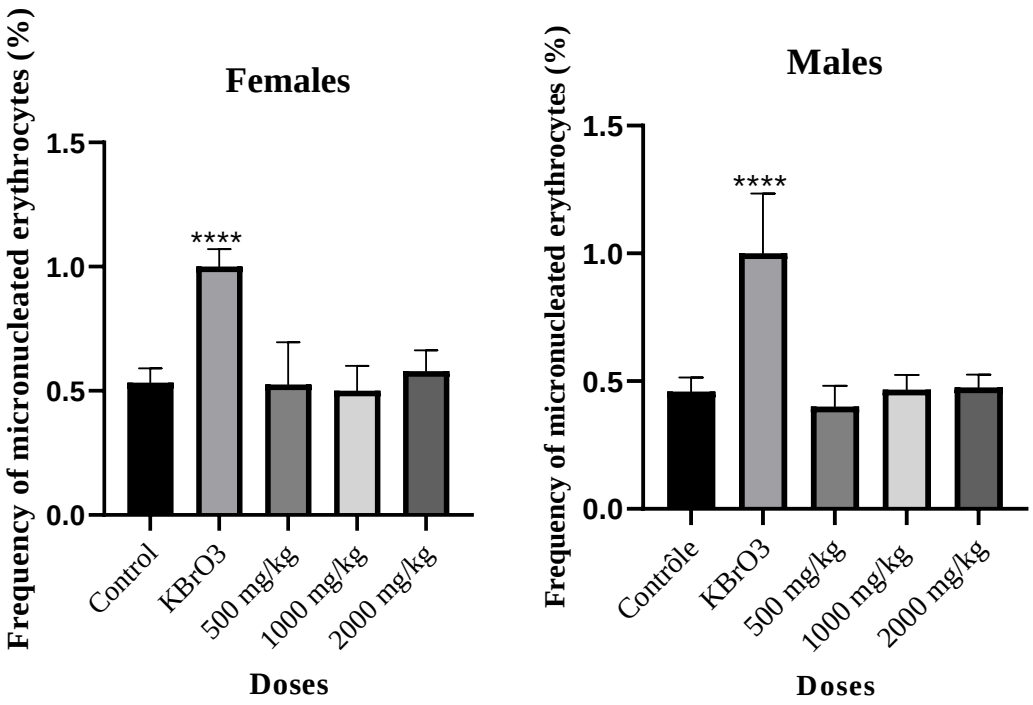
Analysis of the data on the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) also indicated that there was no significant difference ($p > 0.05$) between the negative controls and the test batches for both sexes. However, a very significant difference ($p < 0.01$) was observed between the positive controls (treated with potassium bromate) and the batches treated with the extract.

Analysis of data on the frequency of micronucleated erythrocytes in male and female mice from the test batches compared to the negative controls indicated that there was no significant difference ($p > 0.05$) between the negative controls and the test batches that received the aqueous extract of *P. biglobosa* bark in both sexes. However, a very significant difference ($p < 0.01$) in the frequency of micronucleated erythrocytes was observed between the positive control groups treated with potassium bromate and the test groups treated with the aqueous extract of *P. biglobosa* bark.

The data are shown in Table VII for the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE), and in Figure 4 for the frequency of micronucleated erythrocytes.

Table VII: Number of polychromatic erythrocytes and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in control mice and mice treated with the aqueous stem bark extract of *Parkia biglobosa*.

	Sex	Control	KBrO3	500 mg/kg	1000 mg/kg	2000 mg/kg
PCE	**M**	87.40 ± 10.48	179.00 ± 11.25	69.00 ± 2.45	74.67 ± 6.11	69.00 ± 13.14
	F	66.67 ± 11.02	139.60 ± 29.34	57.75 ± 8.66	72.80 ± 11.80	70.20 ± 3.27
PCE/NCE (%)	**M**	9.64 ± 1.28	22.16 ± 1.66	7.45 ± 0.28	8.11 ± 0.71	7.46 ± 1.54
	F	7.19 ± 1.26	16.44 ± 3.92	6.14 ± 1.00	7.92 ± 1.39	7.60 ± 0.38



Legend: ****p<0.001

Figure 4: Frequency of micronucleated erythrocytes (%) in male and female mice controls and male and female mice treated with the aqueous stem bark extract of *Parkia biglobosa*.

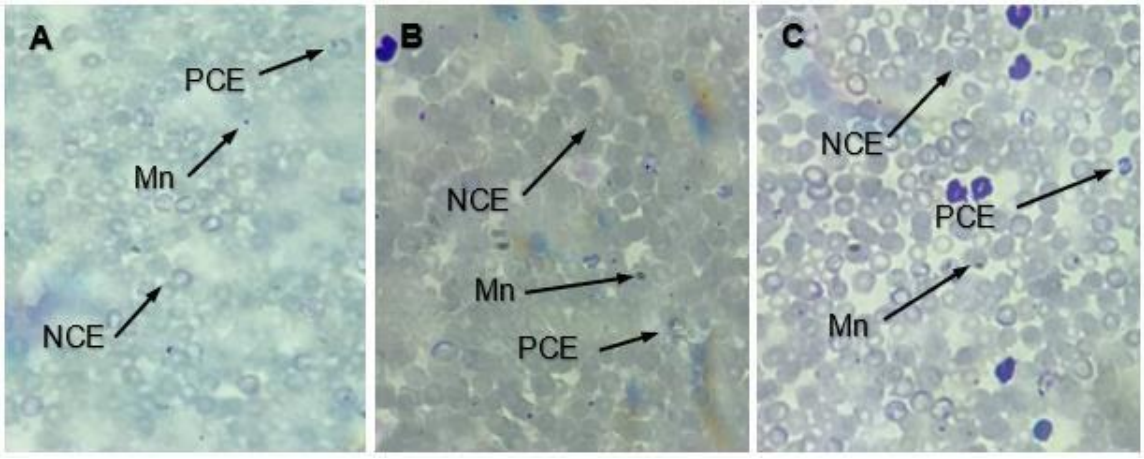


Figure 5: Microscopic images of slides for the evaluation of the mutagenicity of the aqueous stem bark extract of *Parkia biglobosa*: A = negative control; B = positive control; C = test group dose 2000 mg/kg bw.

4. DISCUSSION

P. biglobosa is a plant widely used for the treatment of various diseases. The bark of this plant, for example, is used to treat diseases such as ulcers, bilharzia, malaria, diarrhea, violent

colic, vomiting, infertility, venereal diseases, Guinea worm, edema, rickets, hemorrhoids, leprosy, hookworms, jaundice, and hypertension.[15] Hence, there is an interest in this part of the plant in phytochemical, pharmacological, and toxicological studies to better understand and control its uses. The phytochemical characterization and toxicological evaluation of the aqueous extract of the bark of the *P. biglobosa* trunk, which were the subject of this study, contribute to this understanding of the plant and to ensuring its safe use by the population.

Phytochemical analysis is very important for evaluating the possible medicinal uses of a plant and for determining the active ingredients responsible for its known biological activities.[16] The chemical groups sought were tannins, saponosides, triterpenes and sterols, flavonoids, alkaloids, coumarins, and anthracenosides. There were two main reasons for focusing on these specific groups: first, because the literature reported the presence of some of these groups in extracts from parts of P. biglobosa; and second, because some of these chemical groups are known for their pharmacological and toxicological properties. For example, the authors report the presence of saponins, tannins, flavonoids, terpenes, phenols, sterols, isoquinoline alkaloids, and indole alkaloids in the leaves of *P. biglobosa*.[5,17] The presence of chemical groups such as tannins, triterpenes and sterols, saponosides and flavonoids in the aqueous extract of *P. biglobosa* bark has been reported by several authors.[18,19] In addition, chemical groups such as alkaloids have been investigated because they are known to be toxic to humans and animals. Knowing the presence of a chemical group in an extract and understanding its biological mechanism of action would help clarify the extract's effects, particularly the toxicological effects of interest to this study that may result from its use. The phytochemical characterization of the aqueous extract of *P. biglobosa* bark in this study identified chemical groups, including flavonoids, tannins, triterpenes, sterols, and saponosides. All these chemical groups are considered potentially toxic to organisms exposed to them in the literature.

Although numerous studies have confirmed the safety of flavonoids, their potential toxicity remains a notable area of ongoing research. Their toxicity involves carcinogenicity and mutation, liver and kidney toxicity, influence on thyroid and reproductive function, and disruption of intestinal flora.[20] The harmful effects of sterols on the human body are produced by their excess in the body. Phytosterols can lead to complications such as hemolysis, carcinogenesis, and decreased absorption of vitamins and carotenoids.[21] Extracts of plant and fungal origin containing triterpenes have been studied for many years for their

potential cytotoxicity against neoplastic cells.[22] Hydrolysable tannins are potentially toxic to ruminants. Pyrogallol, a hepatotoxin and nephrotoxin, is a product of the degradation of hydrolysable tannins by ruminal microbes.[23] A study by Diwan et al. on the effects of saponin on mortality and histopathological changes in mice reported a median lethal dose (LD_{50}) of 200 mg/kg body weight. Histological changes limited to the small intestine, liver, and kidneys were also reported. The changes observed in the small intestine included hemorrhages and mucosal erosions. In addition, hepatic and renal lesions resulting from necrosis of liver cells and renal tubular cells were reported.[24]

Given the potentially toxic profiles of the chemical groups detected in the extract and the dose-dependent nature of the toxic effects associated with products containing them, an experimental toxicological assessment was necessary to establish the extract's safety or toxicity.

The acute oral toxicity of the aqueous stem bark extract of *Parkia biglobosa* was evaluated in female Wistar rats to determine any adverse effects that may result from short-term exposure to the extract over a 24-hour period. During this assessment at a dose of 2000 mg/kg body weight, no mortality or major toxic effects were observed. Administration of the extract did not affect weight gain in the treated rats compared with the control group. The lethal dose 50 (LD_{50}) was therefore estimated to be greater than 2000 mg/kg body weight. Builders et *al.* reported an LD_{50} for the aqueous stem bark extract of *Parkia biglobosa* to be greater than 5000 mg/kg body weight.[18] Ahmed et *al*., in their study of the acute toxicity and hepatoprotective effect of the aqueous extract of *P. biglobosa* bark in Wister albino rats, also determined an LD_{50} greater than 5000 mg/kg body weight.[25] These data therefore suggest that *P. biglobosa* extract has low toxicity, since the LD_{50} was greater than 2000 mg/kg body weight. The low toxicity obtained could explain why *P. biglobosa* has been used by traditional medicine practitioners without any reported cases of mortality due to toxicity[18] and has a widespread use in various ethnotherapeutic interventions.

The subacute toxicity assessment conducted over 28 days at doses of 250, 500, and 1000 mg/kg in male and female Wistar rats did not result in any mortality or major visible toxic effects during the trial period. Neither daily water nor food consumption was affected by the extract in treated rats, as no significant differences in either were observed between treated and control rats. However, weight loss was observed in females treated with the extract at doses of 250 and 500 mg/kg from the third week onwards. A reduction in weight gain was

also observed in females from the second week onwards and in males from the third week onwards compared to the controls. This suggests that administration of the aqueous stem bark extract of *P. biglobosa* may affect weight gain in rats at high doses and with repeated administration. These results do not corroborate those of Ibrahim et *al*., who reported that rats treated with different doses of the extract (1000 mg/kg, 500 mg/kg, and 250 mg/kg) showed a significant increase in body weight compared with controls.[26] These results also do not corroborate those of Builders et *al*., who reported no adverse effects on the body weight of rats treated with the aqueous extract of P. biglobosa bark up to 5000 mg/kg.[18] However, this discrepancy in the effect of the aqueous extract of *P. biglobosa* bark on weight gain between the two studies cited above and this study could be explained by differences in the chemical composition of the various extracts. Indeed, Builders et *al.* and Ibrahim et *al*. reported the absence of flavonoids in their extracts, whereas they were detected in this study. However, numerous studies have shown that flavonoids can effectively inhibit obesity and related metabolic disorders. They help to limit weight gain and stabilise metabolism, and act by reducing fat mass.[27,28] The reduced weight gain in the treated rats compared with the controls could therefore be explained by the extract's anti-obesity activity.

The evaluation of the relative weight of isolated organs revealed no significant differences in the liver, lungs, heart, and kidneys between the control group and the groups treated with different doses. Macroscopic examination of these organs also revealed no morphological differences between control rats and those treated with the extract. This suggests that treatment of rats with the aqueous stem bark extract of *P. biglobosa* at the evaluated doses did not cause any visible macroscopic changes in these organs. However, it has been reported that a dose of 5000 mg/kg of the aqueous stem bark extract of *P. biglobosa* can cause a significant increase in kidney weight and severe histopathological changes in the kidneys and liver.[18] This means that at high doses, the aqueous stem bark extract of *P. biglobosa* could cause liver and kidney toxicity.

However, assessment of the relative weight of the spleen revealed a decrease compared with the controls and the groups treated at 1000 mg/kg in both sexes. However, no morphological differences in this organ were observed by macroscopic examination. Given that a change in the relative weight of an organ may indicate a physiological adaptation or a toxic effect, but does not in itself indicate a deleterious effect,[12] a reservation was made regarding the incrimination of the extract for this decrease in relative weight observed for the spleen. Also,

due to variability caused by small size or physiological factors unrelated to treatment, and the lack of correlation with other results, some pharmaceutical studies consider the weight of certain organs to be of limited value.[29] Finally, Builders et *al.* reported no significant differences in the relative weight of the spleen in rats treated with the aqueous stem bark extract of *P. biglobosa* at doses up to 5000 mg/kg.[16]

The evaluation of biochemical parameters revealed no significant differences in serum concentrations of creatinine, total cholesterol, total protein, phosphate, chlorine, sodium, potassium, and ALT between control and extract-treated rats in both sexes. This suggests that administering the extract did not cause damage to the organs responsible for regulating these biochemical parameters in rats treated at the evaluated doses. However, decreases in serum calcium concentration in male rats treated with 500 mg/kg, and serum AST concentration in male rats treated with 1000 mg/kg, compared to control rats, were observed. However, these results do not corroborate those for organ damage, particularly the liver for AST. Liver damage suggests an increase in serum AST concentration.[30] Although a decrease in serum calcium may be linked to renal impairment,[31] the absence of a significant difference in serum creatinine levels between control rats and treated rats contradicts the hypothesis of hypocalcemia due to renal impairment.. In the absence of evidence of renal and hepatic impairment, these decreases in, calcium and AST concentrations could be linked to uncontrolled mechanisms or factors unrelated to the rats' intake of the extract.

Hematological parameters were not evaluated in this study due to technical limitations. Although very limited, the literature reports that the aqueous stem bark extract of *P. biglobosa* does not cause significant changes in hematological parameters at doses up to 5000 mg/kg b.w. For example, Builders et *al.* reported that there were no significant changes in globular volume, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, monocytes and eosinophils, total leukocyte count, differential leukocyte count, erythrocytes, leukocytes, and platelets in all treated groups. The doses evaluated in their study were 1000, 3000, and 5000 mg/kg bw.[18]

The study of the mutagenic power of the aqueous stem bark extract of *P. biglobosa*, conducted in NMRI mice at doses of 500, 1000, and 2000 mg/kg body weight, aimed to evaluate the extract's ability to induce chromosomal damage by forming micronuclei in polychromatic erythrocytes. The micronucleus test in mice is the most widely used and best-validated in vivo genotoxicity test for assessing the genotoxic potential of chemicals and/or

radiation.[32] Chromosomal damage manifests itself in the formation of micronuclei in polychromatic erythrocytes in the bone marrow and erythrocytes in the peripheral blood.[33,34]

The results of this study showed no significant difference in the number of micronuclei in peripheral blood between mice treated with the aqueous stem bark extract of *P. biglobosa* and negative controls. However, a significant increase in the frequency of micronucleated erythrocytes in the peripheral blood of mice treated with $KBrO_3$, the positive control, was observed compared with the negative control. A significant difference in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was also observed between the negative controls and mice treated with $KBrO_3$. However, no significant difference in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was observed between mice treated with the aqueous stem bark extract of *P. biglobosa* and negative controls.

These results show that, at the doses used in this test, the aqueous stem bark extract of *P. biglobosa* has no mutagenic effect on the peripheral blood cells of mice. However, no literature data were found on the evaluation of the mutagenic potential of *P. biglobosa* bark extract for comparison with those obtained in this study.

4. CONCLUSION

This study aimed to determine the phytochemical profile by screening for specific chemical groups and to evaluate the acute and subacute toxicity and mutagenicity of the aqueous stem bark extract of *Parkia biglobosa*, used as the active ingredient in a prototype anti-hemorrhoidal phytomedicine in Burkina Faso. The chemical groups detected in the extract were flavonoids, sterols, triterpenes, saponosides, and tannins. The oral toxicity results showed that, at the tested doses, the aqueous stem bark extract of *P. biglobosa* did not cause any major toxic effects in the animals. However, because the detected chemical groups exhibit toxicological and pharmacological properties, the aqueous stem bark extract of *P. biglobosa* could be of interest for further studies to support its safety or efficacy in traditional uses.

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