

Original Research Article

Evaluation of the cutaneous toxicity of the aqueous extract of the aerial parts of *Mitracarpus scaber* (Rubiaceae) in rats (*Rattus norvegicus* strain Wistar)

Abstract

Mitracarpus scaber is a known plant in the Ivorian pharmacopoeia where it is used in various ways for the treatment of different diseases and ailments, including skin problems. The present study aims at evaluating the cutaneous toxicity of the aqueous extract of the aerial parts of *M. scaber* in the Wistar rat following exposure to a single dose or repeated doses. In the acute dermal toxicity experiment, a total of six (6) female rats were divided into two (2) groups, with three (3) rats per group. While a total of 32 male and female rats were divided into four (4) groups, each group consisted of eight (8) rats, four (4) males and four (4) females in the subacute dermal toxicity experiment. For the acute dermal toxicity study, rats in one group received a single application of the extract at the dose of 2000 mg/kg body weight (bw) on the first day of the experimental period. While the rats in the subacute study received topical application of the extract at the doses of 200, 400 and 800 mg/kg bw once a day for 28 days. The various applications were made to the dorsal shaved area of the skin. Throughout the respective fourteen (14) and twenty-eight (28) day study periods, all rats were monitored for any changes in physical appearance and behavior that might occur due to the toxic effects of the plant. No mortality or abnormal physical appearance was observed, and physiological and behavioral changes were not observed in all rats in either study. Body weight, kidney and liver weights, and hematology and biochemistry results did not show significant differences ($p > 0.05$) between all groups in the subacute study. Similarly, histopathological examinations of the liver, kidney and skin revealed no structural alterations. This study suggests that topical application of the aqueous extract of the aerial parts of *M. scaber* does not induce acute and subacute adverse effects on the skin or systemic toxicity in Wistar rats.

Keywords: Toxicity, dermal, *Mitracarpus scaber*, *Rattus norvegicus*

Introduction

Mitracarpus scaber Zucc is one of the species of the Rubiaceae family that has gained popularity as a remedy for dermatitis. It is a herb with a puberulent stem, where the leaves are lanceolate and hairless. The genus *Mitracarpus* is known by four (4) synonyms: *M. scaber* (Zucc), *M. hirtus* (L), *M. vertillatus* (Schmach and Tonn) and *M. villosus* (Sw). The whole plant is used topically for the treatment of dermatoses. The leaves are used as a body rub to treat fever, the leafy branches are used in the treatment of fungal infections [1,2]. Due to its application to the skin, assessment of the acute and subacute dermal toxicity effects of the plant is important to ensure its safety before it can be safely used for medicinal purposes. OECD guideline 402 defines acute dermal toxicity as adverse effects observed shortly after dermal application of a single dose of a test substance [3]. For subacute dermal toxicity the OECD guideline 410 considers a test substance to be toxic when potential health risks are likely to result from repeated dermal exposure to the test substance over a limited period of time [4]. Studies have shown that some plants applied to the skin have induced allergic and/or granulomatous dermatitis [5,6]. Therefore, the present study was conducted to evaluate the dermal toxicity of the aqueous extract of the aerial parts of *M. scaber* in rats, which could be used later to establish safe and optimal doses to treat skin problems.

1. Material and methods

Material

1.1.1. Plant material

Fresh aerial parts of *M. scaber* were collected in Daloa, west-central Côte d'Ivoire, during September 2020. The plants were shade dried at room temperature for 15 days, and powdered using an electric grinder (Retsch, model GM 300). The powder was used for the preparation of the total aqueous extract.

1.1.2. Animal material

Rats, *Rattus norvegicus* of Wistar strain male and female ranging in weight from 120 to 160 g were used for these studies. The rats were individually placed in polypropylene plastic cages. The animals were housed in the animal house of the Ecole Nationale Supérieure (ENS) with controlled conditions involving these parameters: temperature ($22\pm 2^{\circ}\text{C}$), humidity ($55\pm 10\%$) and lighting (12 hours light/dark).

1.2. Methods

1.2.1. Aqueous extraction

The total aqueous extract of the aerial parts of *Mitracarpus scaber* was prepared following the method of Yapo et al. [7]. Leaf powder (Fifty grams, 50 g) was macerated in one (1) liter of distilled water in a blender (NASCO BL 9295-A), the mixture was mixed for 10 minutes. The resulting homogenate was filtered twice through white cloth and then five times through absorbent cotton. The filtrate obtained was evaporated at 50°C in the oven.

1.2.2. Preparation of the animals

Skin preparation was performed under general anesthesia. Rats were anesthetized with thiopental, the injection was done intramuscularly. The furs in the dorsal region were trimmed with an electric clipper and shaved with a razor blade. The shaved area was applied with either a plant extract or distilled water. This manipulation was done on the day before each study.

1.2.3. Acute toxicity

Acute dermal toxicity was conducted in female rats using the OECD Protocol 402 limit test at 2000 mg/kg body weight [3]. Prior to the application of the extract, six (06) rats consisting of two (02) batches of three (03) rats, were deprived of food, but not water for eighteen (18) hours. The animals were weighed, and then the test substance was applied to the animals of the first batch, the animals of the second batch were treated with distilled water, the solvent that was used to dissolve the dry extract. The extract was applied once and uniformly to the exposed skin surface from the back to the flank, at least 10% of the total body surface. After application of the aqueous extract of the leaves of *M. scaber*, the animals were observed regularly for the first twenty-four (24) hours and then once a day for fourteen (14) days. The body weight of each rat was carefully monitored before the study and once a week during the study.

1.2.4. Subacute toxicity

The subacute dermal toxicity of the aqueous extract of the aerial parts of *M. scaber* was determined according to the OECD 410 clinical test guideline using doses of 200; 400 and 800 mg/kg body weight (bw) for a period of twenty-eight (28) days [4]. A total of thirty-two (32) rats, including 16 males and 16 females, were used to conduct this study. The rats were divided into four (4) batches of eight (8) animals each. The animals of the first batch, received distilled water. The animals in batches 2; 3 and 4, received locally the aqueous extract of the aerial parts of *M. scaber* at doses of 200; 400 and 800 mg/kg bw respectively. Each rat was weighed before the study, once a week for twenty-eight (28) days and on the day of sacrifice. After the twenty-eight (28) days of topical application, on day twenty-nine (29th) blood samples were collected in EDTA tubes to analyze hematological parameters and in non-EDTA tubes to prepare serum for biochemical analysis. Rats were sacrificed by decapitation and organs such as heart, liver, kidneys, lungs, spleen, thymus and adrenal glands were quickly removed, weighed and preserved in 10% formalin for histopathological analysis.

1.2.4.1. *General evaluation of the general signs and behavior of the rats*

All rats were monitored daily for mortality, changes in fur, eyes, mucous membranes, behavior (salivation, tremors, convulsions, diarrhea and lethargy) and respiration during the acute and subacute toxicity study.

1.2.4.2. *Determination of hematological parameters*

Hematological analysis was performed using an automated hematology analyzer (Cell-Dyn®, 3700, Abbot, USA).

1.2.4.3. *Determination of biochemical parameters*

Biochemical analyses were performed using a fully automated biochemical analyzer (HITACHI 704 R).

1.2.4.4. *Histopathology*

Skin, liver and kidney samples collected and fixed in 10% formalin for 48 hours were sliced and placed in plastic cassettes to be dehydrated in different **ethanol** baths of increasing degree 80% for 2 hours, **90%, 96% and 100%** for one hour each. Then, the organs were thinned for 20 min in three toluene baths for four (4) hours. In addition, the organs were impregnated in two **paraffin** baths for five (5) hours in the oven at 70°C. After this step, they were embedded in **paraffin** at room temperature. The tissue samples were then cut to 5 µm thickness with a microtome (Leica RM2125 RTS). Mounting was performed on glass slides. Dewaxing was then performed by toluene bath exchange, after which rehydration was performed using different dilutions of 100%, 90% and 80% ethanol for fifteen (15) minutes each. The tissue sections were then rinsed with tap water and stained with hematoxylin and eosin (H&E). Finally, the slides were covered with coverslips using EUKITT adhesive. All images were taken with a digital camera (Amscope MD130) mounted on a microscope (Olympus CX31, Co., Tokyo, Japan). Amscope software was used to capture the images.

1.2.5. Data analysis

The obtained data were statistically analyzed using GraphPad Prism software, version 8. Values were expressed as mean \pm error on the mean for different parameters. Repeated measures analysis of variance (ANOVA) tests were performed to compare differences in data between and within groups.

2. Results and discussion

2.1. Results

2.1.1. Acute dermal toxicity in rats

2.1.1.1. *General sign and behavior of rats*

Topical application of the aqueous extract of the aerial parts of *M. scaber* at the single dose of 2000 mg/kg body weight (bw) did not show significant changes or impairments in the behavior, breathing pattern, posture, skin, fur, skin effects such as irritation, ulceration, and rash, sensory nervous system responses of the animals. The extract at the dose of 2000 mg/kg bw did not reduce food and water consumption in treated rats (**Figure 1 and 2**). No mortality was observed during the 14 days of observation after application of the extract.

Figure 1 : Average volume of water consumed by rats after topical application at a single dose of 2000 mg/kg bw of the extract

Figure 2 : Average amount of food consumed by rats after topical application of a single dose of 2000 mg/kg bw of the extract

2.1.1.2. *Effect of aqueous extract of the aerial parts of M. scaber on the weight of animals after application of a single dose of 2000 mg/kg bw*

Figure 3 shows the change in body weight of rats after topical application of the aqueous extract of the aerial parts of *M. scaber*. The aqueous extract did not produce a significant ($P > 0.05$) change in body weight compared with the control.

Figure 3 : Change in body weight of rats after a single dose of 2000 mg/kg bw of the extract

2.1.2. Subacute dermal toxicity in rats

2.1.2.1. *Animal behaviour*

Rats treated for 28 days with topical application of aqueous extract of *M. scaber* at doses of 200, 400 and 800 mg/kg bw showed no change in the general physical appearance and somatomotricity of the rats during the treatment period. No evidence of tremors, convulsions, salivation, diarrhea, coma, or abnormal behaviors such as self-mutilation or walking backwards was observed in the rats.

2.1.2.2. *Effect of aqueous extract of aerial parts of M. scaber on body weight of animals*

Figure 4 shows the weight changes of the animals during the 28 days of treatment. No significant differences were noted in the average weight gains in treated rats compared to controls.

Figure 4 : Effect of the aqueous extract of the aerial parts of *M. scaber* during 28 days of topical application

2.1.2.3. Effects of aqueous extract of the aerial parts of *M. scaber* on organ weight

Macroscopic examination of the different organs after 4 weeks of topical application of the aqueous extract of the aerial parts of *M. scaber* showed no morphological changes in the organs of the rats treated with 200, 400 and 800 mg/kg body weight compared to the organs of the untreated rats either for color or texture. No significant difference was observed between the weights of the different organs of the treated rats and those of the control rats (**Table 1**).

Table 1 : Organ weights of animals treated with aqueous extract of the aerial parts of *M. scaber* during 28 days of topical application

Doses	Relative weight of organs (g)						
	Heart	Kidneys	Liver	Adrenal glands	Thymus	Spleen	Lungs
Control	0,383±0,053	0,538±0,083	2,513±0,383	0,023±0,003	0,150±0,024	0,240±0,018	0,733±0,069
200 mg/kg bw	0,337±0,013	0,482±0,014	2,305±0,101	0,028±0,003	0,153±0,013	0,225±0,016	0,740±0,062
400 mg/kg bw	0,338±0,017	0,472±0,018	2,117±0,124	0,020±0,000	0,107±0,005	0,212±0,020	0,762±0,069
800 mg/kg bw	0,323±0,018	0,452±0,028	2,070±0,210	0,015±0,003	0,102±0,005	0,175±0,015	0,590±0,060

Values are means ± MSE (n=8/lots), p>0.05

2.1.2.4. Effects of the aqueous extract of the aerial parts of *M. scaber* on biochemical parameters

2.1.2.4.1. Serum lipid concentration

Topical application of the extract caused a highly significant (p<0.001) and highly significant (p<0.01) decrease in serum triglyceride levels at 800 and 400 mg/kg body weight, respectively, of the treated animals compared with the control lot. No significantly change was noted in serum total cholesterol level of treated compared to control (**Table 2**)

Table 2 : Effect of aqueous extract of the aerial parts of *M. scaber* on serum concentration of lipid and renal parameters during 28 days of topical application

Parameters	Unit	Doses			
		Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
Total cholesterol	g/L	0,698±0,009	0,705±0,019	0,698±0,034	0,710±0,029
Triglycerides	g/L	1,058±0,051	1,022±0,052	1,053±0,073	0,957±0,038

Values are means ± MSE (n=8/lots), p>0.05.

2.1.2.4.2. Serum concentration on renal parameters

Renal function parameters, such as urea and creatinine, showed no significant changes (Table 3).

Table 3 : Effect of aqueous extract of the aerial parts of *M. scaber* on serum concentration of renal parameters during 28 days of topical application

Parameters	Unit	Doses			
		Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
Urea	g/L	0,238±0,021	0,238±0,015	0,258±0,023	0,285±0,026
Creatinine	mg/L	4,000±0,000	4,500±0,224	4,500±0,224	4,500±0,342

Values are means ± MSE (n=8/lots), p>0.05

2.1.2.4.3. Serum concentration of liver parameters

No significant differences ($P > 0.05$) were found in the liver function enzymes tested, such as ALT and AST. There was no significant difference in total protein, conjugated and total bilirubin compared with the control group (Table 4).

Table 4 : Effect of aqueous extract of the aerial parts of *M. scaber* on serum concentration of liver parameters during 28 days of topical application

Parameters	Unit	Doses			
		Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
ASAT	UI/L	204,800±25,330	221,300±15,240	265,200±16,970	249,700±29,470
ALAT	UI/L	61,750±20,890	55,330±7,986	60,500±3,622	49,170±2,725
Total Bilirubin	mg/L	5,350±0,278	5,752±0,603	6,567±0,512	6,583±0,409
Conjugated bilirubin	mg/L	1,078±0,285	1,022±0,077	1,610±0,643	1,063±0,085
Total proteins	g/L	79,500±3,476	83,830±0,833	83,170±1,922	84,170±2,971

Values are means ± MSE (n=8/lots), p>0.05.

ALAT : alanine aminotransférase, ASAT : aspartate aminotransférase

2.1.2.5. Effects of the aqueous extract of the aerial parts of *M. scaber* on hematological parameters

No significant changes ($p > 0.05$) were observed in erythrocyte, platelet, and leukocyte values (Table 5 and 6).

Table 5 : Effect of aqueous extract of the aerial parts of *M. scaber* on erythrocyte parameters

Parameters	Unit	Doses			
		Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
Red blood cells	$\times 10^{12}/L$	7,785 \pm 0,125	8,128 \pm 0,169	7,993 \pm 0,216	7,718 \pm 0,183
Hemoglobin	g/dL	13,150 \pm 0,119	13,870 \pm 0,194	13,870 \pm 0,255	13,450 \pm 0,211
Hematocrit	L/L	42,650 \pm 0,375	44,080 \pm 0,543	44,670 \pm 1,155	42,150 \pm 0,744
MCHC	g/dL	30,83 \pm 0,413	31,47 \pm 0,243	31,07 \pm 0,358	31,93 \pm 0,376
MCV	fl	54,80 \pm 0,618	54,28 \pm 0,652	55,97 \pm 1,228	54,68 \pm 0,888

during 28 days of topical application

Values are means \pm MSE (n=8/lots), $p>0.05$.

MCHC : Mean Corpuscular Hemoglobin Concentration

MCV : Mean corpusculaire volume

Table 6 : Effect of aqueous extract of the aerial parts of *M. scaber* on leukocyte and platelet parameters during 28 days of topical application

Parameters	Unit	Doses			
		Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
White blood cells	$\times 10^9/L$	23,010 \pm 1,808	22,510 \pm 1,017	24,330 \pm 0,614	22,670 \pm 0,372
Neutrophils	$\times 10^9/L$	2,665 \pm 0,129	2,410 \pm 0,130	3,262 \pm 0,354	2,367 \pm 0,144
Lymphocytes	$\times 10^9/L$	8,205 \pm 0,371	8,578 \pm 1,249	8,325 \pm 0,750	8,725 \pm 0,283
Monocytes	$\times 10^9/L$	5,378 \pm 0,582	4,303 \pm 0,592	3,725 \pm 0,352	3,642 \pm 0,463
Eosinophils	$\times 10^9/L$	0,593 \pm 0,048	0,555 \pm 0,041	0,502 \pm 0,040	0,523 \pm 0,033
Basophils	$\times 10^9/L$	1,668 \pm 0,170	1,208 \pm 0,161	1,367 \pm 0,149	1,193 \pm 0,194
Blood platelets	$\times 10^9/L$	844,000 \pm 72,380	731,800 \pm 61,540	884,000 \pm 64,080	740,200 \pm 53,190

Values are means \pm MSE (n=8/lots), $p>0.05$

2.1.2.6. Histopathological examination

Figure 5 shows the histopathological examination of the liver, kidney and skin of rats from the different batches, no significant differences were observed. None of the organs of the rats treated with the extract showed any alteration of the cell structure or other negative effects when examined under the microscope with different magnifications.

Figure 5: Representation of histological sections of organs from different batches

No significant pathological lesions were observed in all groups during the study of the cutaneous toxicity of the aqueous extract of the aerial parts of *Mitracarpus scaber* (Haematoxylin-Eosin staining; magnification x100)

Ep : Epidermis ; **Fp** : Hair follicle ; **F** : Fibroblasts ; **F** : Fur

Hc : Hepatic cells; **Vc** : Centrobular vein; **S** : Sinusoid

Dct : Distal contoured tube; **Pct** : Proximal contoured tube ; **G** : Glomerulus; **Us** : Urinary space

2.2. Discussion

Toxicological evaluation after repeated exposures are required by regulatory agencies to characterize the toxicological profile of any substance [4]. *In vivo* studies are required to determine the signs of toxicity as well as the correct dose range of these plants [8]. The present study was conducted to investigate the dermal toxicity of the aqueous extract of the aerial parts of *M. scaber*. However, the results of this study revealed no signs of toxicity and death observed throughout the experimental period in the acute and subacute toxicity studies. No rats exposed topically to the aqueous extract of the aerial parts of *M. scaber* exhibited toxicity at the single dose of 2000 mg/kg bw in the acute dermal study or at doses of 200, 400 and 800 mg/kg bw in the subacute dermal study. The LD₅₀ is greater than 2000 mg/kg bw, therefore the extract is classified as category 5 non-toxic according to the Globally Harmonized System of Classification and Labelling of Chemicals. Rats were monitored daily for signs of toxicity or mortality until day 14 of the acute study and until day 28 of the subacute study. During this monitoring period, the rats showed no obvious signs of distress. No symptoms of toxicity were noted and there were no deaths. The clinical abnormality is one of the key important observations to detect toxicity effects on the organs of treated animals [9]. No rats showed significant behavioral changes. In addition, physical appearance, such as skin, fur, and eyes, was normal. In addition, adequate feeding and water consumption are essential for a therapeutic safety study. Adequate feeding has a significant effect on the status of the animals and on the measured effects of the test drugs [10]. In these studies, food and water consumption remained normal. This lack of effect on appetite means that there was no disruption of carbohydrate, protein or fat metabolism levels [11]. According to Raza et al. [12], changes in body weight are indicators of adverse effects of drugs and chemicals. These changes become relatively more significant if body weight loss exceeds -10%. During the fourteen (14) days of observation or twenty-eight (28) days of topical application of the extract, the weight gain was similar whether in the batches of rats given the extract at the doses of 2000; 800; 400 or 200 mg/kg bw compared to the controls.

The relative weight of the organ is fundamental in diagnosing whether the organ has been exposed to the deleterious effects of a chemical [13]. According to Dybing et al. [14], the liver, kidney, and spleen are the major organs affected by the metabolic response caused by a toxicant. In this study, the general appearance of organs collected at the end of subacute toxicity test from the control and treated batches was normal. There was no significant difference in relative organ weights between control and treated rats. Local application of aqueous extracts of the aerial parts of *M. scaber* had no adverse effects on organ weights.

The analysis of biochemical parameters allows for the assessment of toxic effects on tissues, and more specifically on the kidneys and liver. Some enzymes and proteins, such as transaminases, bilirubin and proteins, can be used as indicators of the hepatocellular effects of a chemical [15]. Others such as creatinine, urea, and uric acid, are considered biomarkers of nephron function [16]. In this study, no statistically significant differences were observed in all

biochemical parameters. The hematopoietic system is sensitive to toxic compounds. It is an important indicator of physiological and pathological status in animals and humans [17]. Toxic substances can directly affect mature cells in the bloodstream or indirectly or directly affect precursor cells in the bone marrow, and this can cause an abnormal reduction or increase in the number of cells in the hematopoietic system. The results show that the extract did not induce any significant alteration of the hematological indices, which suggested that the aqueous extract of the aerial parts of *M. scaber* has no systemic deleterious effect in the treated animals during the twenty-eight days of treatment. These results are similar to those of Reduan et al [18], who applied the ethanolic extract of the leaves of *Melastoma malabathricum* (Malastomataceae) to the skin of rats at doses of 250, 500 and 1000 mg/kg body weight. A 1999 study by Ajagbonna [19] and his team revealed that the normal range of hematological parameters can be altered by ingestion of toxic plants.

Histopathology can be used to confirm alterations in tissue structure due to toxicity. Histopathological examination remains the gold standard method for assessing treatment-related pathological changes in tissues and organs [20]. The histopathological evaluation performed for this study to determine subacute dermal toxicity indicated that the aqueous extract of the aerial parts of *M. scaber* did not cause structural damage in the morphology of the liver, kidney and skin. The histopathological results confirm the results of the hematological and biochemical analyses. In this study, liver histology revealed normal hepatocytes. The portal and central vein, bile duct and hepatic artery showed no structural alterations in both control and treated rats. No necrosis, inflammatory reaction or fibrosis was observed. These results are in agreement with Ali et al. [9], the results of the latter did not reveal tissue changes in the liver of the rats. Kidney histology revealed no adverse effects of the aqueous extract of the aerial parts of *M. scaber* applied to the skin of the animals for 28 days. The glomeruli and Bowman's capsules were all normal. Microscopic examination of the skin of the rats treated with the extract did not indicate any changes in the skin layers at the epidermis, dermis, and hypodermis level compared with the control rats.

Conclusion

The results suggest that topical application of the aqueous extract of the aerial parts of *Mitracarpus scaber* at a single dose of 2000 mg/kg bw, or at doses of 200; 400 and 800 mg/kg bw repeated daily for 28 days does not cause allergic skin reaction and systemic toxicity *in vivo*. The extract is therefore classified as non-toxic by the dermal route.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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