ejpmr, 2024, 11(7), 112-118

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYTOCHEMICAL SCREENING AND DIURETIC EFFECTS OF AN AQUEOUS EXTRACT OF EUPHORBIA HIRTA (EUPHORBIACEAE) LEAVES IN WISTAR RATS

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Article Received on 15/05/2024 **Article Revised on 05/06/2024** **Article Accepted on 25/06/2024**

ABSTRACT

Euphorbia hirta (Euphorbiaceae) is a medicinal plant used in traditional medicine to treat a wide range of number of pathologies, in particular hypertension. Therefore, the present study aims to evaluate the biotolerance and diuretic effect of an aqueous extract of *Euphorbia hirta* (Euphorbiaceae) leaves on wistar rat. The acute toxicity study was conducted according to the Organisation for Economic Co-operation and Development (OECD 423) guideline. In this study, MFEH was administered from 1000 mg/kg bw, 2000 mg/kg bw and followed by 5000 mg/kg bw. Rats were observed for toxic signs at 24 h and the next 14 days. For the MFEH diuretic effect, water-overloaded rats were treated separately (n=6) with increasing doses (100 to 600 mg/kg bw) of *Euphorbia hirta* aqueous extracts (MFEH), Furosemide (Furo) and Hydrochlorothiazide at 10 mg/kg bw. Blood sample of each rat and excreted urine was collected (measured for 24 hours) for various biochemical analyses. MFEH at a single dose of 5000 mg/kg bw showed no lethal effects. MFEH exhibits superior urinary excretion kinetics than that induced by Furosemide and Hydrochlorothiazide. These findings are in agreement with the traditional claim for use *Euphorbia hirta* leaves as diuretic agent.

KEYWORDS: *Euphorbia hirta; Urine; Electrolyte; Furosemide; Hydrochlorotiazide, edema.*

INTRODUCTION

Medicinal plants are used throughout the world in general and in Africa in particular in primary health care in primary health care.[1] According to the World Health Organization (WHO), approximately 65-80% of the world's population in developing countries lack access to primary due to poverty and lack of access to health services. Due to poverty and lack of access to modern medicine. The use of medicinal plants for various health problems in Africa is a cultural choice, but it is also poverty and the high cost of modern medicines.[2] According to WHO, nearly 6377 plant species are used in Africa, of which over 4000 are medicinal plants, accounting for 90% of traditional medicine in Africa.[3] Unfortunately, in developing countries, the effects of plants are still empirically, i.e. without the slightest scientific evidence.[4] Many plant species used for skin care contain multiple active ingredients and have numerous active ingredients and have numerous therapeutic effects. They are used in phytotherapy and traditional medicine.[5] Some contain chemical compounds that have an effect on arterial hypertension. For this African pharmacopoeia generally uses plants with diuretic properties.[6] These include *Euphorbia hirta*, a plant in the Euphorbiaceae family. This plant is cultivated in Côte d'Ivoire. In traditional Beninese medicine, *Euphorbia hirta* (Euphorbiaceae) is reputed to treat treatment of gastroenteritis.[7] The various parts of *Euphorbia hirta* have been traditionally used since antiquity for the treatment of numerous pathologies.[8] Previous pharmacological studies have shown that *Euphorbia hirta* exerts several pharmacological effects. The whole plant is renowned for its diuretic properties.[9] These effects, reported by traditional healers, could give *Euphorbia hirta* leaves may give diuretic and antihypertensive properties. The use of a diuretic should help reduce oedema and correct high blood pressure arterial hypertension.[10] The aim of this work is to determine the phytochemical compounds present in aqueous extract of *Euphorbia hirta* leaves, assess his pharmacological effects and highlighting its diuretic power.

MATERIALS AND METHODS

PLANT MATERIAL

The strain of *Euphorbia hirta* (Euphorbiaceae) was collected in Adzopé (Yakassé, Côte d'Ivoire). The plant is authenticated by a botanical expert (Professor Ake-Assi).

Conserved at the *Centre National de Floristique* (CNF) of the UFR-Biosciences of the University Felix Houphouet-Boigny, Abidjan, Côte d'Ivoire.

PREPARATION OF AQUEOUS EXTRACT

Fresh *Euphorbia hirta* leaves are air dried away from direct sunlight. They are then crushed in a micro mill (Culatti® MFC, Allemagne). Ten grams (10g) of the powder is added to hexane and left to macerate for 24 hours. The resulting solution is carefully filtered through absorbent cotton and Wattman filter paper. The filtrate collected in a flask is then evaporated under vacuum at 60°C using a rotary evaporator (Rotavpor Buchi®) and then oven-dried at 50 ± 5°C. The result is a fine powder, perfectly soluble in water is an aqueous extract of *Euphorbia hirta* (MFEH). The residual moisture content is expressed as 0.165±0.025%. The various MFEH solutions are prepared using a solution of sodium chloride (NaCl) 0.9 % solution.

EXPERIMENTAL ANIMALS

Healthy adult Wistar albino rats weighing between 150 and 250 g are used in these experiments. The animals are housed in plastic cages at room temperature and cages and subjected to a light/dark cycle. They are fed ad libitum with pellets, dry bread, corn, and dried fish and watered ad libitum with tap water. All animal experiments have been carried out in accordance with EU guidelines (2007/526/CE).

EXPERIMENTAL DESIGN

Acute Toxicity Study

The acute toxicity study of *Euphorbia hirta* aqueous extract was carried out in accordance with Organization for Economic Cooperation and Development (OECD 423). Animals are fasted for 3 to 4 hours before the start of the experiment. Three groups of albino wistar rat are treated with a single dose at different dosage levels rats are used in this study. The control group (Batch 1) rats are treated with normal saline; Batch 2 rats receives a starting dose of MFEH (1000 mg/kg bw), Batch 3 rats receives a starting dose of MFEH (2000 mg/kg bw) and the Batch 4 rats receives a starting dose of MFEH (5000 mg/kg bw). All animals are observed every 30 minutes for 24 hours, after which they are placed in an incubator. For 14 days after extract administration. Attention is paid to clinical signs such as changes in body weight, skin and capillary changes, somato-motor activity and behavior, followed by various other manifestations such as snacking.

Identification of phytochemical compounds of MFEH

Specific reagents were used to identify the various phytochemical compounds present in the extract specific reagents. The chemical composition of the reagents depends on the nature of the phytochemical compound to be characterized. Sterols and polyterpenes are detected in the aqueous extract using the Liebermann reaction. The presence of flavonoids in the aqueous extract was highlighted the cyanidine test. The presence of alkaloids in the aqueous extract was Bouchardat, Dragendorff and Valser Mayer reagents. Stiasny's reagent to characterize catechic tannins. Quinonic substances are sought in aqueous extracts using Borntraëgen reagent. The presence of saponins is revealed, the presence of alkaloids in the aqueous extract is detected using using Bouchardat (iodo-iodide reagent) and Dragendorff (iodo-bismuth reagent; iodo-bismuthate reagent).

Evaluation of the diuretic activity of MFEH

Experiments are being carried out to validate the use of *Euphorbia hirta* as a diuretic in the treatment of hypertension, as claimed by traditional traditional healers. The experiments were carried out under the same conditions with two pharmacological diuretics (Furosemide and Hydrochlorothiazide). Thirty (30) rats weighing between 186 and 250 g were used to study the comparative effects of aqueous *Euphorbia hirta* leaf extract and the two reference diuretics on urinary excretion. The rats were fasted 16 hours before the start of the test. They were then divided into five batches six animals each, as follows a water overload of 50 ml/kg bw is administered to each animal before any other test substances.

- Batch 1 (Controls): receives water overload 50 ml/kg bw + 0.9% NaCl at a rate of 1 ml/kg bw
- Batch 2: receives water overload (50 ml/kg bw) + Furosemide (Furo), a reference diuretic At 10 mg/kg bw
- Batch 3: receives fluid overload (50 ml/kg bw) + Hydrochlorotiazide (HCTZ) a reference diuretic at 10 mg/kg bw
- Batch 4 and 5: receive water overload (50 ml/kg bw) + respective doses of 200 and 400 mg/kg bw respectively of MFEH.

Animals are immediately put in metabolic cages after being administered by the different substances. Urine was then collected every two hours for 24 hours. At the end of 24 hours, a 2 milliliter sample of each urine is taken and placed in a urine box and stored at -20°C for biochemical analyses. At the end of the observations, the blood is collected by caudal sampling using a dry tube. The blood is then centrifuged in a refrigerated centrifuge at 3000 rpm for 10 minutes. The serum, separated from elements, is collected in aliquot tubes for assay of sodium, potassium, chlorine, calcium, urea and creatinine.

STATISTICAL ANALYSIS

Values are expressed as mean ± standard deviation (m ± esm). GraphPad prism 7 software is used for statistical analysis and graphical representation. The differences between treatments are determined using the analysis of variance (ANOVA) the Turkey-Kramer multiple comparison test. A difference is considered statistically significant when $P < 0.05$.

RESULTS

ACUTE TOXICITY ASSESMENT

Oral administration of *Euphorbia hirta* leaf macerate (MFEH) at doses of 1000, 2000 and 5000 mg/kg bw in rats, did not induce any behavioral disturbances or mortality. Non-lethality due to MFEH at the maximum dose of 5000 mg/kg bw indicates that its LD_{50} is well above this value. The globally harmonized classification places the extract in category 5 and is non-toxic by the oral route. MFEH with an LD_{50} greater than 5000 mg/kg bw is therefore non-toxic when administered orally (**Table I**).

TABLES

Table I: Percentage of rat mortality after MFEH single dose oral administration.

Batchs	Doses (mg/kg bw)	Number of Animals	Number of deaths	Number de survivors	Mortality (%)
1	NaCl 9 ‰ (1ml/kg bw)	3	0	3	0
2	1000	3	0	3	0
3	2000	3	0	3	0
4	5000	3	0	3	0

Table II: *Euphorbia hirta* phytochemical screening.

Compounds		Reagents being tested	MFEH
Sterols and polyterpenes		Liebermann	+
Polyphenols		Chlorure ferrique	++
Flavonoids		Cyanidine	+
Tanins	**Catechiques**	Stiasny	-
	Gallic		-
Quinones		Bornstraegen	+
Alkaloids	**Bouchardat**	Dragendorff and Valser-Meyer	+
	Dragendorff		+
Saponosides		FoamTest	+

***(+) :** Presence **; (-) :** Absence*

MFEH: Aqueous Extract of Euphorbia hirta (*Hephorbiaceae*) leaves.

Table III: Urinary electrolyte concentrations in Wistar rats 24 hours after administration of MFEH.

		FURO	HCTZ	MFEH mg/kg bw	
Parameters	**Controls**	**10 mg/kg bw**	**10 mg/kg bw**	**200**	**400**
Na^+(mmol/l)	142±5.97	225.3±2.48****	185±1.46****	211.2±4.68****	162.3±2.70**
K^+(mmol/l)	25.97±1.21	31.9±1.37**	25.43±0.84	50.64±1.06****	36.30±1.01****
Cl^-(mmol/l)	181.7±4.59	219.3±3.273****	229.3±5.68****	246.5±4.73****	205.5±2.82**
Ca^{2+} (mmol/l)	0.34±0.01	0.36±0.01	0.17±0.01****	0.43±0.01****	0.37±0.01

*Values are expressed as means followed by the standard error of the mean (m ± sem); n = 6 rats per batch. Significant difference: **p < 0.01; ***p < 0.001 ****p < 0.0001 compared with control; MFEH: Aqueous Extract of Euphorbia hirta leaves; FURO: Furosemide; HCTZ: Hydrochlorothiazide.*

Table IV: Plasma electrolyte concentrations in Wistar rats 24 hours after MFEH and reference substances administration.

Parameters	Controls	Furo	HCTZ	MFEH mg/kg bw	
		10 mg/kg bw	**10 mg/kg bw**	**200**	**400**
Na^+ (mmol/l)	137±1.09	127±1.89**	139.3±0.91	126 ±1.43**	123.2±2.91***
K^+ (mmol/l)	5.2±0.29	5.9±0.18	5.28±0.26	7.05±0.17****	6.36±0.19**
Cl^- (mmol/l)	107.7±1.87	106.3±2.20	102.5±2.33	93±2.745**	95.33±2.40**
Ca^{2+}(mmol/l)	99±1.46	99±1.59	101±1.31	88.83±1.83**	91.50±1.64*

*Values are expressed as means followed by the standard error of the mean (m±sem); n = 6 rats per batch. Significant difference: * p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 compared with control;MFEH: Aqueous Extract of Euphorbia hirta leaves ; Furo: Furosemide;*
HCTZ: Hydrochlorothiazide.

FIGURES

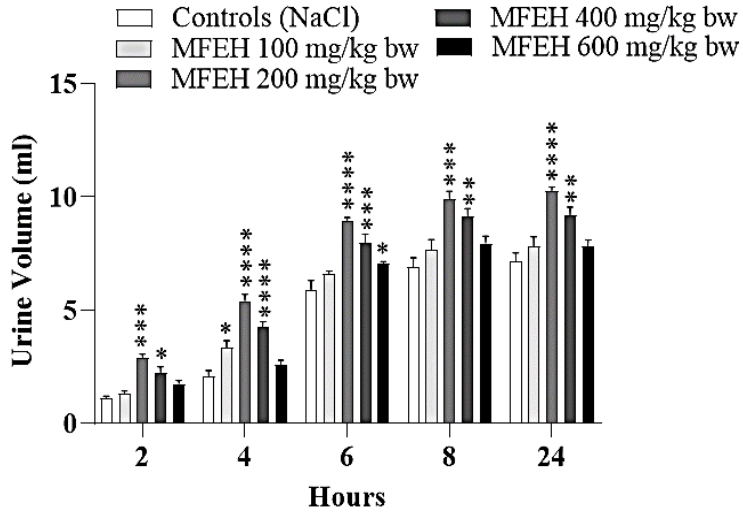

Figure 1: Dose-response effects of MFEH administration after 24 hours in Wistar.

*Values are expressed as means followed by the standard error of the mean (m ± sem); n = 6 rats per batch. Significant difference : *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 compared with control lots;MFEH: macerated leaves of Euphorbia hirta.*

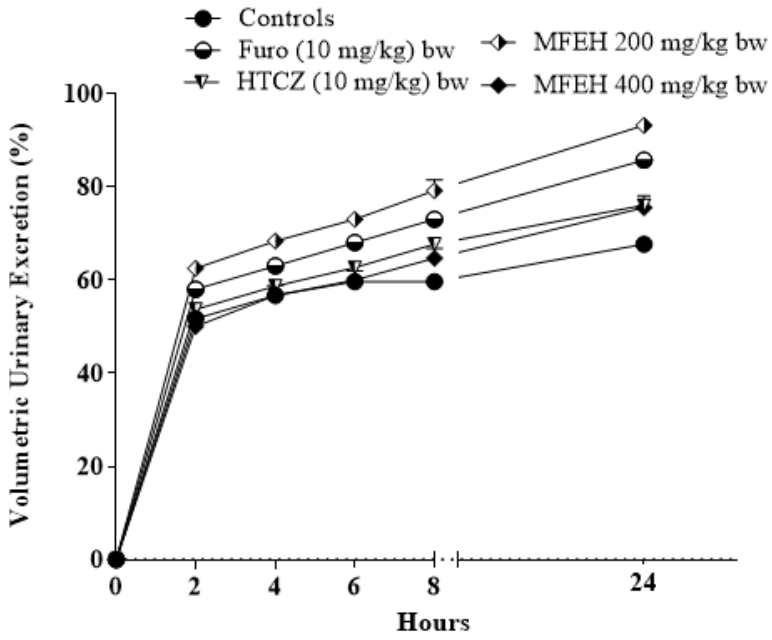

Figure 2: Variation curves for volumetric urinary excretion (VUE) in Wistar rats after administration of increasing doses of MFEH for 24 hours.

*Values are expressed as means followed by the standard error of the mean (m±sem); n = 6 rats per batch. Significant difference: *p < 0.05; **p < 0.01; ***p < 0.001 compared with control lot. MFEH: MFEH: Aqueous Extract of Euphorbia hirta leaves; VUE: Volumetric Urinary Excretion; Furo: Furosemide; HCTZ: Hydrochlorothiazide.*

PHYTOCHEMICAL COMPOUNDS IN THE AQUEOUS EXTRACT OF MFEH

Phytochemical composition of *Euphorbia hirta* aqueous leaf extract Phytochemical screening revealed saponosides, quinones, alkaloids, sterols, polyterpenes, polyphenols, flavonoids and reducing compounds (**Table II**).

DIURETIC ACTIVITY OF AQUEOUS EXTRACT OF EUPHORBIA HIRTA LEAVES ON WISTAR RAT

Dose-response effect of aqueous extract of *Euphorbia hirta* leaves on wistar rat urinary volume

Oral administration of the aqueous extract of *Euphorbia hirta* leaves (MFEH) at doses of 100, 200, 400 and 600 mg/kg bw, causes an increase in urinary volumes (UV) for all doses but significant ($p < 0,01$; $p < 0,0001$) for doses of 200 and 400 (**Figure 1**). The recorded data is 7.82±0.4 ml, 10.28±0.16 ml, 9.2±0.35 ml and 7.85±0.25 ml respectively in rats treated with doses of 100, 200, 400 and 600 mg/kg bw of MFEH compared to 7.16 ± 0.36 ml for the control batch.

Comparative effect of the aqueous extract of *Euphorbia hirta* leaves and reference substances on volumetric urinary excretion on wistar rat

The volumetric urinary excretion (VUE) induced by the various doses of substances administered is very significant ($p < 0,01$). Volumetric urinary excretion induced by MFEH at a dose of 200 mg/kg bw after 24 hours was relatively high compared with furosemide (Furo) and hydrochlorothiazide (HCTZ). The values obtained were 93.25±0.62%, 85.67±1.38% and 76±2.03% respectively (**Figure 2**).

Effects on urinary electrolytes concentration on wistar rats

After 24 hours, urinary electrolyte (Na^+, K^+, Cl^- and Ca^{2+}) excretions induced by MFEH were relatively higher than those induced by Furosemide (Furo) and Hydrochlorothiazide (HCTZ) (**Table III**). MFEH induced a urinary Na^+ excretion of 211.2±4.68, while Furo and HCTZ induced a urinary Na^+ excretion of 211.2±4.68 mmol/l. Furosemide (Furo) and hydrochlorothiazide (HCTZ) induced 225.3±2.48 mmol/l and 185±1.46 mmol/l respectively. Urinary K^+ levels measured were 50.64±1.06 mmol/l; 31.9±1.37 mmol/l and 25.43±0.84 mmol/l respectively for MFEH (200 mg/kg bw), Furo and HCTZ. Urinary Cl^- concentrations were 246.5±4.73 mmol/l; 219.3±3.273 mmol/l and 229.3±5.68 mmol/l respectively for MFEH (200 mg /kg bw), Furo and HCTZ. MFEH induced urinary Ca^{2+} excretion of 0.43±0.01 mmol/l, while Furo and HCTZ induced 0.36±0.01 mmol/l and 0.17±0.01 mmol/l respectively.

Effects on plasma electrolytes concentration on wistar rats

After 24 h, plasma electrolyte (Na^+, K^+, CL^- and Ca^{2+}) excretions induced by MFEH were relatively lower than those induced by Furosemide (Furo) and Hydrochlorothiazide (HCTZ) (**Table IV**). MFEH induced a plasma Na^+ excretion of 126±1.43 mmol/l, while Furo and HCTZ induced a plasma Na^+ excretion of 1.43±1.2 mmol/l, Furo and HCTZ induced 127±1.89 mmol/l and 139.3±0.91 mmol/l respectively. Plasma K^+ levels measured were 7.05±0.17 mmol/l, 5.9±0.18 mmol/l and 5.28±0.26 mmol/l respectively for MFEH (200 mg/kg bw), Furo and HCTZ. Plasma Cl^- concentrations were 93±2.74 mmol/l, 106.3±2.20 and 102.5±2.33 mmol/l respectively for MFEH (200 mg /kg bw), Furo and HCTZ. MFEH produced a plasma Ca^{2+} excretion of 88.83±1.83 mmol/l, while Furo and HCTZ induced 99±1.59 mmol/l and 101±1.31 mmol/l respectively.

DISCUSSION

Regarding acute toxicity, oral administration of *Euphorbia hirta* leaf macerate (MFEH) at doses of 1000, 2000 and 5000 mg/kg bw in rats, did not induce any behavioral disturbances or mortality. The globally harmonized classification places the extract in category 5 and is non-toxic by the oral route.[11] These results are similar to those Kwan *et al.*, who showed the methanolic extract of *Euphorbia hirta* leaves, administered orally at a dose of 5000 mg/kg bw induced no signs of toxicity.[13] In addition, the toxicity level of MFEH in acute administration is similar to those obtained with several plant species used in traditional Ivorian medicine, notably *Piper umbellatum* (Piperaceae), *Rawvolfia vomitoria* (Apocynaceae), *Pseudarthria hookeri* (Fabaceae).[14.15.16] Phytochemical screening of MFEH revealed the presence of saponosides, quinones, alkaloids, sterols, polyterpenes, polyphenols, flavonoids and reducing compounds, Saponosides, quinones and alkaloids are also present in most of the plants traditionally used to treat hypertension, including *Melissa officinalis* and *Cymbopogon citratus*.[17] The cardiovascular of these plants are attributed to the compounds they contain. Sterols and polyterpenes have antiseptic, anthelmintic, disinfectant, anti-inflammatory, antipyretic and analgesic properties.[18] Sterols are also precursors of sex and adrenocortical hormones. Polyphenols are recognized as antioxidants, anticancer agents, anti-inflammatories and vasorelaxants and play an important role in preventing degenerative and cardiovascular diseases.[19] Flavonoids in particular are antioxidant substances active in maintaining blood circulation. They help increase the production of nitric oxide in blood platelets, which limits clot formation by preventing the formation of platelets from clumping together, thus helping to prevent atherosclerosis.[20] Similarly, Ojewole has shown that flavonoids have hypotensive and anti-hypertensive properties.[21] Flavonoids have diuretic. anti-inflammatory, antihemorrhoidal and relax vascular endothelium.[22] They also have diuretic properties. by inhibiting Ca^{2+}. anion and water reabsorption.[23] Flavonoids also possess hepato-protective properties.[24] The diuretic effect of aqueous extract of *Euphorbia hirta* leaf is carried out by measuring urinary volume, volumetric urinary excretion and the urinary and serum content of electrolytes (Na^+, K^+, Cl^-, Ca^{2+} and Mg^+). Evaluation of the dose-response effect of MFEH resulted in pharmacological doses of 200 and 400 mg/kg bw respectively volumes of 10.28±0.16 ml and 9.2±0.35 ml, whereas Furo and HCTZ induced urine volumes of 8.56±0.38 ml and 7.8±0.08 ml after 24 hours. This could mean that MFEH

possess a diuretic effects than these reference substances. These results are similar to those obtained.[12] Indeed, this author showed that the aqueous extract of the leaves of *Piper umbellatum* (Piperaceae) at doses of 200 and 400 mg/kg bw are highly effective on diuresis in rats. and its effects would be more diuretic than furosemide and hydrochlorothiazide. MFEH. administered at a dose of 400 mg/kg bw. produced a significant increase in urine volume in rats. Bhavin and collegue showed that aqueous extract of *Pergularia daemia* (Apocynaceae) at a dose of 400 mg/kg bw, are effective on diuresis in rats.[25] The Volumetric urinary excretion, 24 hours after administration of MFEH is similar to that of furosemide and hydrochlorothiazide. This diuretic effect of MFEH is confirmed by a EUV of 93.25±0.62% at a dose of 200 mg/kg bw. These results are similar to those obtained by Yao *et al*.[26] In indeed. these authors reported with the ethanolic fraction of *Phyllanthus amarus* (Phyllanthaceae) at doses of 40 mg/kg bw. Higher than those of Furosemide and Hydrochlorothiazide. MFEH is more diuretic than furosemide and hydrochlorothiazide. However, the elimination of the water overload observed with MFEH at a dose of 200 mg/kg bw is significantly greater than that recorded in controls and in rats treated with furosemide and hydrochlorothiazide.

CONCLUSION

The aqueous extract of *Euphorbia hirta* leaves contains mainly saponosides. quinones. alkaloids. sterols. polyterpenes. polyphenols. flavonoids and reducing compounds. A study of the dose-response effect of aqueous leaf extract *Euphorbia hirta* leaves on urinary excretion showed that the highest urinary excretion 200 mg/kg bw orally. This excretion is associated with significant urinary elimination of electrolytes. MFEH exhibits urinary excretion kinetics similar to those induced by Furosemide and Hydrochlorothiazide. These beneficial effects in healthy rats appear to be due to the action of certain secondary metabolites. The action of certain secondary metabolites acting alone or in synergy, justifying in part the traditional use of this plant in the treatment of arterial hypertension and edema.

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