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Research Article

Diuretic effects and subacute toxicity of *Trema orientalis* Linn leave extract in wistar rats

Tokou Labite Kpoti *, Togbenou Komlatsè, Dosseh Kossivi, Idoh Kokou, Kpatcha Tchazou, Agbonon Amégnona

Laboratory of Physiology and Pharmacology (LAPHYPHAR), Department of Physiology, Faculty of Sciences, University of Lome

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*Address for Correspondence:

Tokou Labite Kpoti, Laboratory of Physiology and Pharmacology (LAPHYPHAR), Department of Physiology, Faculty of Sciences, University of Lome

Abstract

Trema Orientalis is a medicinal plant used in the treatment of cardiovascular diseases including hypertension. The present study was undertaken to investigate phytochemical compounds, diuretic activity and subacute toxicity of the hydromethanolic leaf extracts of Trema orientalis. Phytochemical analysis carried out through color and precipitation reactions revealed the presence of many metabolites. The measurement of diuretic activity carried out by saline overload with 0.9% NaCl at a dose of 50 mL/kg in wistar rats showed that the hydromethanolic extract of *Trema orientalis* leaves had diuretic activity at a dose of 200 mg/kg of body weight. However, this diuretic activity was relatively low compared to the action of the reference molecule, furosemide. The study of the subacute toxicity of the extract took place over 28 days. No significant difference was recorded in the body weights during the 28 days of the study. Likewise, the weight of the organs collected after sacrifice of the rats and their macroscopic aspects did not show any significant difference with those of the control group. The extract increased the count of white and red blood cells as wells as platelets, practically at the two doses used and caused a decrease in ALT at the dose of 500 mg/kg. The extract could have a beneficial effect like a boosting immunity and would not present any toxicity at these doses.

Keywords: phytochemical, diuretic, subacute toxicity, *Trema orientalis*

INTRODUCTION

For centuries plants have provided mankind with useful; sometimes life-saving drugs [1] . However, the use of plants by many populations is sometimes done empirically and does not take into account the new requirements of modern therapy. Several laboratory research are nowadays undertaken, according to experimental models, to scientifically confirm the therapeutic potential of these medicinal plants.

Many studies such as the one conducted by [2] have shown that synthetic diuretics can have physiological side effects that could prevent their effective use. The search for new forms of treatment is therefore necessary to compensate for the use of these synthetic drugs. Research carried out on medicinal plants conceals an interesting potential for the discovery of new substances of a diuretic nature, since plants possess a reservoir of bioactive molecules originating from their secondary metabolites.

Many populations, especially in Africa, consider medicinal plants as an affordable and more accessible alternative to the drugs used by modern medicine. There is therefore a real interest in knowing the different effects of these extracts on the body when taken for a long time.

Our study focused on *Trema orientalis* a medicinal plant used in Togo. In the traditional and popular medicine of several countries, this plant is sometimes used alone or in combination with other plants as a treatment for several diseases. The leaf decoction of *Trema orientalis* mixed with leaves of *Bidens pilosa*, *Citrus aurantifolia* and unripe pineapple peels, is used in the treatment of jaundice [3] .Trema orientalis infusions are also used as an emmenagogue, to induce abortions [4] . In Togo this plant is used to treat several disease such as hypertension or hepatic insufficiency [5] . A study conducted by Kadissoli and al.,(2012) et al shows vasodilating effects of the hydromethanolic extract of the leaves on smooth muscle structures [6] .

In order to specify and optimize the use of *Trema orientalis* we tried to evaluate the activity of the hydromethanolic extract of the leaves of *Trema orientalis* (HMETO) on the diuresis of wistar rats as well as their subcacute toxicity.

1. MATERIALS AND METHODS

1-1 – Material

1-1-1 - Plant Material

Trema orientalis leaves were collected in Lome in September 2019 and then dried for several days in the laboratory sheltered from the sun and then ground. Then we proceeded to the identification in the herbarium of University of Lome in Togo under the number TOGO 15872. The powdered leaf was used to obtain the extract.

1-1-2 - Animal material

Either sex of wistar rats weighing between 125 and 200 g procured from the Animal House of the physiology and pharmacology laboratory (LAPHYPHAR) of the University of Lome in Togo were used for the study. They were housed in a standard environmental condition and fed with rodent standard diets and water ad libitum. They were evenly distributed into treatment groups and kept in cages.

1-2 Methods

1-2 1 Obtaining hydromethanolic extract of leaves of *Trema orientalis*

Trema orientalis leaves were harvested and dried for a week under air conditioning. After drying, the plants were finely ground. A maceration of 200g of the plant material in 2 liters of methanol/water (70/30; V/V) was carried out to extract the active principles. The mixture obtained is filtered through cotton and filter paper. The extracts obtained were concentrated to dryness under reduced pressure using a brand rotavapor (brand Büchi R 400) at 39°C. The extract obtained was packaged and put in the fridge at 20°C for conservation.

1-2-2 Phytochemical analyses

Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phytoconstituents such as flavonoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, by standard methods of coloration and precipitations [7,8].

1-2-3 Estimation of diuretic and salidiuretic activity

1-2-3-1 Composition of experimental groups and animal preparation in vivo diuretic and natridiuretic and salidiuretic activities

Wistar rats weighed between 150-210 g were fasted for 18 h before each test but with free access to tap water only. Before the experimentation, the animals were conditioned for a few days to receive increasing volumes of water or NaCl 0.9% by intragastric tubing. By way of comparison, experiments are carried out in the same conditions with a synthetic pharmacological diuretic, furosemide, the powdered tablets of which are diluted in the animal's gavage water. For each activity, 8 groups of 5 rats (3 males and 2 females) were formed and each group of rats received a treatment after saline overload of NaCl 0.9% or distilled water at 50 mL/kg of bw.

1-2-3-2 Determination of the diuretic activity

Diuretic activity was estimated using methods described by different authors[9–11].The principle is to take the measurement of urinary excretion in Wistar rats after saline overload. 50 mL/kg of a solution of NaCl 0.9 % were administered to Wistar rats before the various treatments. Groups were then formed as follows:

Group 1 (control): received 25 mL per kg body weight of NaCl 0.9%

Group 2 and 3 (treated): received 100 and 200 mg/kg of bw from the HMETO, respectively

Group 4 (reference): received 20 mg/kg of bw of furosemide

After treatment, Wistar rats from the same group were placed in the cages (1 per cage). For each group, we measured the total volume of urine excreted 6 h after administration.

Excreted Urinary Volume (EUV) was given by the formula:

EUV = VE/VA x 100 (VE = volume excreted, VA = volume administered)

Diuretic activity was estimated according to Kau [12]:

EUV < 80 %.....Antidiuretic activity

EUV between 80-110%.....No diuretic activity

EUV between 110- 130%.....Low diuretic activity

EUV between 130-150%.....Modest diuretic activity

EUV > 150%.....High diuretic activity.

1-2-3-3 Determination of the saludiuretic activity

The principle is the influence of a diuretic on the natriuresis and kaliuresis in animals placed in aqueous overload. Salidiuretic activity was estimated using methods described by authors[9–11] Following groups were constituted

-Group 5 (control) treated with distilled water (25 mL/kg);

-Groups 6 and 7(treated): each received a dose of 100 and 200 mg/kg of HMETO, respectively

-Groups 8 (reference): received furosemide at a dose of (20 mg/kg).

Distilled water at 50 mL/kg were administered to Wistar rats before the various treatments. After treatments, animals from the same group were placed in the metabolic cages (1 per cage). Urine is collected for 6 hours. The kinetics of the hydroelectrolytic eliminations is measured for 6 hours using a Spectrophotometer EBlyte-921F (Direct ion electrode (ISE) measurement method).

1-2-4 Subchronic toxicity

1-2-4-1 Experimental protocol

To assess the subchronic toxicity of HMETO, the repeated-dose oral toxicity method was used [13]. Rats weighing between 150 and 180 g were divided into 3 groups of 6 rats each (3 males and 3 females). Prior to treatment, the rats were carefully examined to diagnose any apparent behavioral abnormalities that would interfere with the test results and conclusions. The extract was dissolved in distilled water and then administered daily at the rate of a single gavage per day for 28 consecutive days at doses of 250 mg/kg (group II) and 500 mg/ kg (group III). The control group (group I) received during the same period distilled water.

Animals were carefully observed each day before and after the gavage sessions throughout the experimental period with respect to mortality, morbidity, changes in posture, changes in hair and eyes, the presence of tearing and behavioral changes.

At the end of each 7 days, the rats were weighed, and the doses were adjusted according to the new weights of the animals. At the end of the 28 days of treatment, the animals were fasted for 12 hours but had free access to drinking water. On the 29th day, rats were anesthetized with ether and the blood was collected by puncture at the level of the retro-orbital vein[14] using capillary tubes.

The blood samples taken were used to assay the hematological and biochemical parameters.

The blood samples for the hematological analysis were collected in tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) as an anticoagulant, while the samples reserved for the biochemical assays were collected in dry tubes and centrifuged at 3000 rpm for 15 mins. After blood sampling, rats were sacrificed by cervical dislocation. Organs such as the liver, kidneys, spleen, lungs, and heart were removed and washed immediately in fresh 0.9% NaCl solution. These

organs were then individually examined macroscopically and then weighed.

1-2-4-2 Measurement of hematological and biochemical parameters

Blood collected in EDTA tubes was used for hematological examinations. Parameters as white blood cells count (WBC), red blood cells count (RBC), hemoglobin level (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count were measured using a hematology analyzer (Mindray BC 6000). The blood samples collected in the dry tubes were used to assay the biochemical parameters. The blood was centrifuged after coagulation, at 3000 rpm for 15 min using an electric centrifuge branded Megafuge 1.0R. Serum was decanted for subsequent assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and others biochemical parameters were measured with a brand spectrophotometer Mindray BS 240 Pro.

2. Statistical analysis

The results are expressed as the mean ± SEM. Data were compared using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism 8.00 software (Graph Pad Software Inc., CA, USA). The results are expressed as mean ± standard error of mean (SEM). The differences observed between the values are considered significant when $P < 0.05$.

3. RESULTS

3-1 Phytochemical analysis

The precipitation and coloring tests showed the presence of most of the compounds except the cyanogenic derivatives. The results have been listed in Table 1.

Table 1: Phytochemical analysis of the hydromethanolic extract leaves of *Trema orientalis*

Chemical compounds class	Tests	Test result
Alkaloids	General test: Dragendorff reagent / Mayer reagent	+
Tannins	Saturation of Na acetate + a few drops of FeCl3, 1% / Stiasny reagent	+
Cardiac glycosids	Reaction of Kedde	+
Tri terpene	Liebermann-Buchard reaction (acetic anhydride-sulfuric acide 50:1)	+
Coumarins	Ammoniac 10% /	+
Saponosides	Foam index (FI) of diluted aqueous decoction (positive if FI ≥ 100, meaning foam height ≥1 cm)	+
Cyanogenic derivatives	Acid picric	-
Mucilages	Chloroform + ammoniac / red ± hight coloration	+
Anthracenosides	Reaction of Bromsträger /ammoniac 25%.	+

+: Presence of chemical compound class; - : Absence of chemical compound class

3-2 Effect of oral administration of Trema orientalis hydromethanolic extract on urine output after saline overload with NaCl 0.9 %

The table 2 shows the variation of urine output after a saline overload. Only the extract at dose of 200 mg/ kg shows a significant variation summarized in figure 1.

Table 2: Effect of oral administration of *Trema orientalis* hydromethanolic extract on urine volume and diuretic index in Wistar rats (n = 5)

	TREATMENTS	VA NaCl (ml)	VE/6h (ml)	E.U.V%	DIURETIC ACTIVITY
Group 1	NaCl 0.9%	10,25 ± 0,32	9,06 ± 0,15	88,39 ^{NS}	NO ACTIVITY
Group 2	100 mg/kg	8,71± 0,58	9,50 ± 0,44	109,07**	NO ACTIVITY
Group 3	200 mg/kg	9,03 ± 0,41	10,68 ± 0,77	118,27***	LOW ACTIVITY
Group 4	Furo 20 mg/kg	8.25 ± 0,32	13,50 ±0,28	163,63****	IMPORTANT ACTIVITY

The comparison was made from NaCl 9 % (control), NS: No significant difference, ** significant difference, ***moderately significant difference, **** highly difference

3-3 Effect of oral administration of *Trema orientalis* hydromethanolic extract on urine output after distilled water overload.

The table 3 shows the variation of urine output after distilled water overload at 50 mL/kg. Only the extract at dose of 200 mg/ kg shows a significant variation

Table 3: Effect of oral administration of *Trema orientalis* hydromethanolic extract on urine volume and diuretic index in Wistar rats (n = 5)

	TREATMENTS	VA ED (ml)	VE/6h (ml)	E.U.V	DIURETIC ACTIVITY
Group 5	Water distilled	9,52 ± 0,58	9,52 ± 0,42	100 NS	NO ACTIVITY
Group 6	100 mg/kg	9,52 ± 0,58	10,02 ± 0,69	105,25 NS	NO ACTIVITY
Group 7	200 mg/kg	9,15 ± 0,49	11,50 ± 0,93	125,68*	LOW ACTIVITY
Group 8	Furo 20 mg/kg	8,91 ± 0,46	14,06 ± 0,54	157,80***	IMPORTANT ACTIVITY

The comparison was made from distilled water (control), NS: No significant difference, * significant difference, **moderately significant difference, *** highly difference

3-4 Effect of oral administration of *Trema orientalis* hydromethanolic extract in kinetic of hydroelectrolytic eliminations

The table 4 shows the kinetic of hydroelectrolytic eliminations of ion sodium, potassium and chloride in treated group with extract was less and not significant compared to the furosemide group.

Table 4: Effect of oral administration of Trema orientalis hydromethanolic extract in kinetic of hydroelectrolytic eliminations

TREATMENTS		Concentration of ions (mmol/L)		
		Na^+ mmol/l	K^+ mmol/l	Cl^- mmol/l
Group 5	Distilled water	16.87 ± 0.68 NS	12.08 ± 0.82 NS	16.72 ± 0.62 NS
Group 6	100 mg/kg	15.45 ± 0.75 NS	13.36 ± 2.86 NS	17.65 ± 2.41 NS
Group 7	200 mg/kg	24.20 ± 3.61 NS	20.24 ± 3.68 NS	27.00 ± 4.50 NS
Group 8	Furo 20 mg/kg	102.17 ± 7.48 ***	40.35 ± 5.64 ***	137.00 ± 5.64 ***

NS: No significant difference, * significant difference, **moderately significant difference, *** highly difference

3-5 Effect of oral administration of hydromethanolic extract of *Trema orientalis* on weight change in rats for 28 days

The curve in figure 2 shows a regular evolution of the weight of the different groups having received the extract and those having received the distilled water. The analysis of the results did not show any significant difference between them.

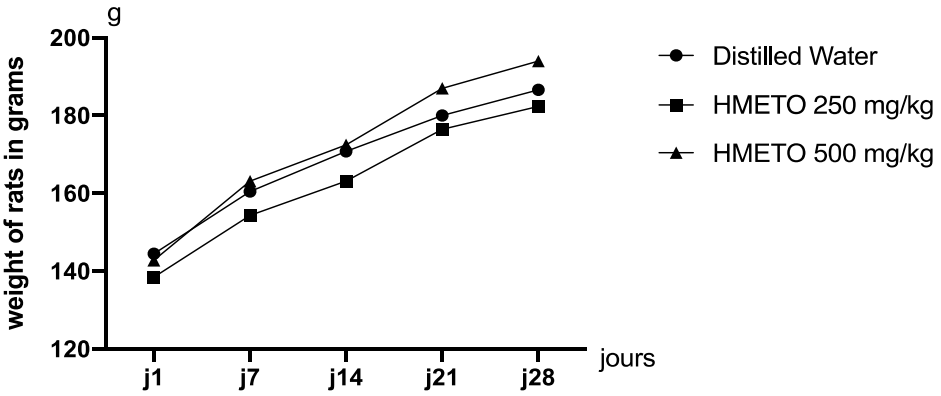

Figure 1: Curve of variation in the weight of rats during the subacute toxicity

3-6 Effect of oral administration of *Trema orientalis* hydromethanolic extract on organ weight

Table 5 gives us a summary of the analysis of the weight of the organs of the rats collected in each rat group after having sacrificed them. The results were not significant.

Table 5: Effect of oral administration of *Trema orientalis* hydromethanolic extract in organ weight

organ	DW	HMETO 250 mg/kg	HMETO 500 mg/kg
Liver	5,15 ± 0,39	5,39 ± 0,34	6,36± 0,46
Spleen	0,65 ± 0,02	0,50 ± 0,03	0,62 ± 0,03
Lung	1,26 ± 0,12	1,02 ± 0,20	1,30 ± 0,08
Heart	0,60 ± 0,08	0,59 ± 0,02	0,59 ±0,04
Kidneys	1,35 ± 0,07	1,03 ± 0,03	1,087 ± 0,03

3-7 Effect of oral administration of Trema orientalis hydromethanolic extract in blood parameters

Table 6 shows the variation of the different blood parameters in different groups. it will be noted here that the extract increased white blood cells count very significantly at both doses and that of platelets at a dose of 500 mg/kg per bw. The extract caused a decrease in MCV in the treated groups as well as an increase in MCHC.

Table 6: Effect of oral administration of hydromethanolic extract of *Trema orientalis* extract on blood parameters

Parameters	DW	HMETO 250 mg/kg	HMETO 500 mg/kg
WBC 10^3/UL	9,24 ±1,15	17,29 ± 1,04**	20,093 ± 2,195****
RBC 10^6/UL	7,30 ± 0,46	8,24 ± 0,30	8,83 ± 0,23**
HGB g/dL	13.47 ± 0,53	15,27 ± 0,46*	15,20 ± 0,07**
HCT %	40,67 ± 1,60	44,62 ± 1,38	44,00 ± 0,31
MCV fL	56,05 ± 1,32	54,17 ± 0,64	49,92 ± 1,05**
MCH pg	18,55 ± 0,26	18,52 ± 0,23	17,27 ± 0,40*
MCHC g/dL	33,15 ± 0,50	34,22 ± 0,06**	34,60 ± 0,14***
PLT 10^3/UL	816,75 ± 74,15	891,00 ± 58,31	1067,83 ± 25,72*
NEU 10^3/UL	2,51 ± 0,39	2,48 ± 0,24	2,96 ± 0,55
EOS 10^3/UL	0,31 ± 0,10	0,32 ± 0,05	0,63 ± 0,10*
BAS10^3/UL	0,00 ± 0,00	0,01 ± 0.00	0,01 ± 0,00
LYM 10^3/UL	5,90 ± 0,66	13,52 ± 1,04**	12,82 ± 2,41*
MON 10^3/UL	0,52 ± 0,08	0,94 ± 0,07*	0,93 ± 0,19*

The comparison was made from water distilled (standard), * significant difference, **moderately significant difference, ***highly difference, **** very highly difference

3-8 Effect of oral administration of Trema orientalis hydromethanolic extract in o blood parameters

Table 7 summarizes the variations of the different biochemical values obtained in the different groups. It should be noted here that most of the parameters did not vary significantly. the differences were perceived at the level of the glycemia and that of the level of ALT. The blood sugar which increased significantly at the dose of 250 and the ALAT level decreased in the group treated with the dose of 500 mg/kg of bw

Table 7: Effect of oral administration of hydromethanolic extract of *Trema orientalis* extract in biochemical parameters

Parameters	DW	EHMTO 250 mg/kg	EHMTO 500 mg/kg
Urea mg/dL	0,37 ± 0.02	0,37 ± 0,03	0,37 ± 0,02
Blood sugar mg/dL	0,48 ± 0,04	0,72 ± 0,10	0,61 ± 0,03
Creat mg/dL	5,20 ± 0,30	5,40 ± 0,20	5,66 ± 0,08
ASAT U/L	140,12 ± 5,40	133,17 ± 6,37	143,92 ± 5,68
ALAT U/L	49,95 ± 1,71	51,55 ± 2,84	42,17 ± 1,45
GGT U/L	1,42 ± 0,22	0,95 ± 0,16	1,45 ± 0,35
ALP U/L	135,78 ± 13,16	113,10 ± 13,43	136,57± 13,23
T Bili mg/dL	1,60 ± 0,05	0,92 ± 0,07	0,89 ± 0,08
D Bili mg/dL	0,88 ± 0,02	0,87 ± 0,06	0,80 ± 0,06
Tot Prot mg/dL	69,77 ± 1,57	70,02 ± 0,50	73,55 ± 3,07
Tot Chol mg/dL	0,54 ± 0,06	0,49 ± 0,02	0,53 ± 0,04
Trigly mg/dL	0,77 ± 0,09	0,84 ± 0,23	0,80 ± 0,11
HDL mg/dL	0,18 ± 0,01	0,20 ± 0,02	0,19 ± 0,01
LDL mg/dL	0,21 ± 0,05	0,12 ± 0,03	0,18 ± 0,02

The comparison was made from distilled water (standard), * significant difference, **moderately significant difference, *** highly difference.

4. DISCUSSION

This work was carried out with the aim of verifying the diuretic potential as well as the subacute toxicity of the extract of *Trema orientalis* because several parts of the plants are used for their medicinal potentials [5]. It would be a good idea to verify some of these properties because the infusion of certain plants like *Portulaca oleraceaa* or *Spondias mombin* have shown that they could have an important diuretic activity [15,16].

Phytochemical screening of HMETO leaves revealed the presence of several molecules such as alkaloids, flavonoids, phenolics compounds, tannins, carbohydrates, glycosides, polysaccharides, proteins alkaloids, steroids triterpenes, flavonoids, tannins, triterpenoids, saponins, sterols. The phytochemical characterization of aqueous extract of leaves of *Trema orientalis* using tubes and thin layer chromatography revealed the presence of flavonoïdes, coumarins, sterols and triterpens, tannins (gallic and cathechic), reducing sugars and saponosides [17]. Several studies have explained that these molecules from the secondary metabolism of plants are responsible for their medicinal activities [18]. Work on the *Orthosiphon stamineus* has shown that some of its flavonoids which could have been isolated could be responsible for the diuretic activity of this plant [19].

Diuretics are pharmacological agents which increase the urinary excretion of sodium and therefore of water. In medicine, diuretic is mainly used to lower blood pressure, reduce the effects of heart failure, peripheral edema, portal hypertension, decrease hyperkalemia and incidentally hypercreatinine [19]. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na+/K+/Cl- cotransport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na+ and Cl- from the body.

The diuretic activity of HMETO was evaluated with furosemide as standard control and saline NaCl 0.9% as negative control. The volume of urinary elimination is summarized in Table 1 which shows the variation in the volumes of urine excreted every hour until the 6h of experiment. At the end of 6 hours, the average amount of urine collected in the positive control furosemide group (20 mg/kg) was very significant (EUV= 163.63%). Only the group having received the extract at a dose of 200 mg/kg presents significant variation of an excreted urinary volume (EUV) in the rats, which was 118,27 %. The extract at this dose increased significantly urinary excretion in rats. The diuretic index at a dose of 200 mg/kg indicates a low diuretic potential for our extract according to kau [12]. The diuretic activity evaluated with furosemide as positive control and distilled water as negative control, shows dose of 200 mg/kg of extract has low diuretic activity (Table 3) which confirmed the results obtained previously. The work of Uddin [20] on the diuretic potential of the methanolic extract of Trema orientalis leaves in mice at a dose of 200 and 400 mg/kg showed a diuretic potential comparable to furosemide. This difference in results can be explained by the fact that our extract contains a lower proportion of secondary metabolites which can contribute to a large increase in urinary output volume. Moreover, the nature of the solvent used, and the form of extraction carried out had an impact on the lower proportion of molecules having a diuretic potential. in our extract. In some cases, the type of solvent used to extraction may not significantly extract the molecule responsible for the activity studied. The capacities of the different *Punica granatum* extracts varied depending on the extraction's solvent and the concentration of extract used [21]. In other cases, the type of extraction carried out can also have an impact on the result obtained depending on whether a maceration or a decoction or an infusion is carried out. Reasearch has shown that *Sarcocephalus latifolius* and *Senna siamea* infusions were the best in terms of diuretic activities, thus explaining their use in folk Beninese medicine [22].

Table 4 shows elimination of ion sodium, potassium and chloride in group treated with extract was no significant compared to the positive control group. Significant increase in Na^+, K^+ Cl^- ion excretion ($p < 0.05$) was observed in treated animals at dose of 200 mg/kg but it was less than furosemide control and higher than the animal group who received distilled water. Compared to the work carried out by Uddin with the methanolic extract of *Trema orientalis* leaves, the elimination of ions in our rats fed with our hydromethanolic extract was lower [20]. This can be explained by the fact that our extract has less diuretic effect on our animals, reducing therefore the elimination of ions in the urine. Moreover, the concentrations of ions Na^+, K^+ Cl^- excreted in the group having taken the extract at dose of 200 mg/kg are lower than the control group, it can be said that the extract, while having a diuretic activity, has restricted the loss of these ions which would lean our hypotheses towards an action comparable to thiazide diuretics. The study conducted by Kasmi showed that the ethanolic extracts of the leaves and barks of *Fraxinus angustifolia* had diuretic effect similar to our extract and seems to be close to thiazide diuretics [23].

This diuretic and saludiuretic effects of hydromethanolic extract of *Trema orientalis* even if low, can be beneficial in the treatment of certain cases of arterial hypertension or the management of certain heart disease by increasing urinary excretion of part of water and sodium in the blood. This will result a decrease in blood volume and therefore a drop in blood pressure.

Effects related to daily oral administration at repeated doses of hydromethanolic extract of *Trema orientalis* were assessed after evaluating behavioral parameters, weight growth, relative weight of organs, blood and biochemical parameters.

Observation of behavior throughout the study period revealed that, regardless of the dose of the extract administered, no behavioral change was observed for 28 days. Toxicity assessment was carried out by observing body or organs weight loss. At the level of curve 1, it can be seen that the weight of the wistar rats has globally increased in a constant manner over time, no matter the batch considered. Although the statistical analysis did not show any significant differences in terms of the variation in weight of the different batches to be considered, curve 1 shows us all the same that the extract at 500 mg/kg led to a more pronounced weight gain in rats. The study conducted with the aqueous extract of the dry leaves of Trema orientalis did not show any toxicity at 2000 mg/kg and would cause weight gain in mice at this dose following an increase in food intake [17]. On the other hand, the extract had no effect on the macroscopic aspect and on the weight of organs such as the liver, spleen, kidneys, heart, and lungs. No significant difference was observed between the weights of the different organs from one batch to the other, as shown by the results in Table 4.

The hematological result of this study summarized in the table 5 reveals that daily administration of hydromethanolic extract leaves of Trema orientalis resulted in increased red blood cell count at 500 mg/kg. Hemoglobin and MCHC were increased at the dose of 250 and 500 mg/kg per body weight. On the other hand, there is a decrease in the rate of MCV, MCH only at the dose of 500. Our extract may contain substances capable of acting on erythropoiesis by borrowing mechanisms to increase the level of erythrocytes. Finally, the decrease in MCV, MCH and the increase in MCHC suggests that the extract restore the size and hemoglobin content of erythrocytes, which is synonymous with cell multiplication and normal hemoglobin synthesis. Hemalatha found that methanolic extract of *Trema orientalis* leaves increased hemoglobin levels and suggested that extracts of T. orientalis may reduce anemia [24]. This same observation was made by Muhammad [25] with *Phyllanthus niruri* leaf extract. The dose of 500 mg/kg of the extract would stimulate more the production of red blood cells which can be used for the management of anemia. Compared to the control, the extract increased the level of platelets and white blood cells in treated rats. The extract thus promotes the production of immune cells, which explains the high level of white blood cells, especially those of eosinophils at a dose of 250 mg/kg and those of lymphocytes and monocytes at two doses 250 and 500 mg/kg. The eosinophils are the immune cells making it possible to fight against the infectious agents of the parasitic type and the lymphocytes are the cells producing antibodies making it possible to fight against foreign elements. In addition to increasing the level of white blood cells platelets are sentinel cells that contribute significantly to anti-infectious immunity [26]. The extract had a stimulating effect on the production of blood platelets, which could give it an immune boosting effect against infections and other diseases affecting immunity. The study conducted by Kasim showed that the bark methanolic extract of *Trema orientalis* had an appreciable hematopoietic effect and that it was non-toxic [27].

Indeed, the heart, the kidneys and the liver are the preferred targets of toxic substances. Once damaged, these organs release their enzymatic or protein contents into the blood. The results of the biochemical parameters in Table 6 suggest that the extract at the study dose did not have a significant impact on the various biochemical parameters of the rats. The extract had a beneficial effect on the liver because the ALAT level decreased at 500 mg/kg, which may suggest a hepatoprotective effect. Our results are similar to those of Sangare [28] who showed that repeated oral administration of the aqueous extract of *Gomphrena celosiodes* for five days at 500 mg / kg led to a decrease in ALT and the ASAT indicating that this extract would be hepatoprotective.

CONCLUSION:

At the end of our study, it appears that the extract of leaves of *Trema orientalis* have therapeutic virtues that can justify their use in traditional medicine. The phytochemical screening made it possible to show the presence of numerous compounds such as: coumarins, flavonoids, oses and holosides and cardiotonic heterosides. We did find a low diuretic activity at the dose of 200mg/kg. The extract does not appear to be toxic. We hope by this work to have marked a starting point towards the development of an improved traditional drug indicated in the treatment of arterial hypertension or certain heart diseases.

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