Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Anthelmintic effect of *Portulaca oleracea* Linne (Portulacacea) against gastrointestinal parasite *Haemonchus contortus* Rudolphi and toxicity screenings

Adamou Ahmadou[1], Issiyakou Haman[1], Francis Nveikoueing[1], Irene Ajonina-Ekoti[2], Toua Vroumsia[1] and Dieudonné Ndjonka[1]

Abstract

Background: The resistance of gastrointestinal worms to synthetic anthelmintics (Levamisole) leads us to highlight the Cameroonian pharmacopoeia. The main objective of the present work is to look for an alternative treatment for haemonchosis, based on active secondary metabolites, from *Portulaca oleracea*, with less or no side effects and accessible to all.

Methods: The *Haemonchus contortus* cycle test was performed at varying concentrations. Levamisole and PBS were the positive and negative controls respectively. Phytochemical screening was performed by standard staining and precipitation methods. Acute and sub-acute toxicity tests of *Portulaca oleracea* EA were performed according to OECD 425 and 407 respectively.

Results: EM at 48 h of incubation inhibited eggs with an LC_{50} of 3.44. There was more larvicidal effect of ME with an LC_{50} value of 3.54 at 48 h incubation. At 24 h of incubation at the final concentration of 1000 µg/mL the anthelmintic effect of EA, ME and levamisole were noted with LC_{50} values of 0.057, 0.096, and 0.069 respectively. Phytochemical screening revealed the presence of some secondary metabolites in EA and ME of *Portulaca oleracea*. The result of the assays shows that ME is richer in total polyphenol (50.884 mg EAG/g DM) and flavonoids (5.688 mg RE/g DM) compared to EA which has (12.998 mg EAG/g DM) and (1.847 mg EC/g DM) respectively. However, there are more tannins in EA (5.688 mg RE/g DM) compared to ME (1.847 mg EC/g DM). The acute and subacute toxicity test showed no toxicity in mice and rats respectively.

Conclusion: In view of the above, *Portulaca oleracea* possesses anthelmintic effects on the parasite *Haemonchus contortus* and is not toxic at the experimental therapeutic dose, which may open a way for the searching of a new anthelmintic drug.

Keywords: *Haemonchus contortus; Portulaca oleracea;* Levamisole; Phytochemical screening; Toxicity.

**Correspondence: Phone: +237 691 677 064 / +237 673 091 894 E-mail address: profndjonka_dede@yahoo.com; ORCID: https://orcid.org/000-0003-1478-6262 (Professor Ndjonka Dieudonné)*

[1]Department of Biological Sciences, University of Ngaoundere P. O. BOX: 454, Ngaoundere, Cameroon; [2]Department of Animal Biology and Physiology, Faculty of Science, University of Bamenda, Bamenda, Cameroon

Other authors:
E-mail: adamouahmadou64@yahoo.fr; ORCID: https://orcid.org/0000-0003-3773-2777 (Adamou Ahmadou); E-mail: issiyakouhaman@gmail.com; ORCID: https://orcid.org/0000-0003-3782-5391 (Issiyakou Haman); E-mail: belngous.lacoste@yahoo.fr ; ORCID: https://orcid.org/0000-0002-8910-0184 (Francis Nveikoueing); E-mail: uajoninai@yahoo.com; ORCID: https://orcid.org/0000-0002-6101-4444 (Irene Ajonina-Ekoti); E-mail: : tvroumsia@yahoo.fr; ORCID: https://orcid.org/0000-0002-5769-6542 (Toua Vroumsia)

Citation on this article: Adamou A, Haman I, Nveikoueing F, Ajonina-Ekoti I, Vroumsia T, Ndjonka D. Anthelmintic effect of Portulaca oleracea Linne (Portulacacea) against gastrointestinal parasites Haemonchus contortus Rudolphi and toxicity screenings. Investigational Medicinal Chemistry and Pharmacology (2021) 4(2):56; Doi: https://dx.doi.org/10.31183/imcp.2021.00056

; © The Author(s). 2021 Open Access This article is available at https://investchempharma.com/

Background

In Africa, Cameroon is one of the most active countries in small ruminant breeding, with an estimated 8.6 million livestock [1]. In the humid tropical zone, this livestock farming is highly affected by gastrointestinal parasites, with losses that can exceed 50 % of production potential [2]. These losses are estimated at about 10 % for milk and 15 % for flesh and are the reasons for the renouncement of small ruminant farming due to the high cost of healing unhealthy animals [3]. The anemia resulting from infestation by ingestion of stage 3 larvae during grazing [4] generates metabolic disorders, leading to the economic non-value of the animal, and even to its death [5]. The main diagnostic feature of haemonchosis is the excessive consumption of blood in the abomasum. Control of these parasitoses has long relied on the use of synthetic anthelmintics [6]. These include benzimidazoles and pro-benzimidazoles, imidazothiazoles and macrocyclic lactones [7]. These treatments are administered frequently and often without prior evaluation of the reality of the parasite risk. In the Imidazothiazoles family, levamisole represents the molecule most widely used in breeding [8]. In general, these various conventional anthelmintic molecules have a number of limitations such as, the malfunctioning and counterfeiting of these drugs, the remoteness and/or inadequacy of human and animal health centers, especially in rural areas, which limit the effective management of public health problems [9]. Because of all these problems, it is necessary to find an alternative treatment based on appropriate medicinal plants, with a strong anthelmintic activity to fight the pathologies caused by these nematode parasites of small ruminants. According to the World Health Organization [10], more than 80 % of African populations used to traditional pharmacopoeia to cope with health problems. So, an ethnobotanical survey was carried (breeders, traditional healers) at dang in Ngaoundere III Subdivision, Adamaoua Region where a certain number of plants have been cited in the treatment of gastrointestinal parasitosis, including *Portulaca oleracea*. However, a screening was done in the laboratory to determine the most effective part (leaves, roots, stems and whole plant) of this plant. The Portulacaceae family, is recognized worldwide for its traditional and pharmacological properties. It can be used to treat burns, headaches, and diseases associated with the gut, stomach, cough, shortness of breath, and arthritis [11]. Such as or including anti-viral [12], anti-bacterial [13] and anti-helminthic [14]. The main objective of this work is to contribute to the searching of an alternative treatment for haemonchosis, based on active secondary metabolites, from *Portulaca oleracea*, with less or no side effects and accessible to all.

Methods

Plant collection and identification

The whole plant (root, stems, leaves and seeds) of *Portulaca oleracea* was harvested in May 2019, precisely in Dang (Adamaoua-Cameroon). The plant was identified by senior lecturer Prof. Pierre Marie Mapongmetsem, a botanist of the University of Ngaoundereand laboratory of Botany and Plant Organisims) and was confirmed at the National Herbarium of the IRAD Yaoundé with identification number n°615 of the herbarium collection n°14491 SRF Cam. The samples were, after washing, dried in the shade for 4 months because of its water content. The parts were crushed and sieved using a 0.4 mm diameter fine mesh sieve [15].

Preparation of Portulaca oleracea extracts

50 g of *Portulaca oleracea* powder was macerated in 500 mL of methanol-distilled water (70: 30, v/v) for 48 h at room temperature. These mixtures were then centrifuged in a Centrifuge (Centrifuge 5804-eppendorf) at 3500 rpm for 10 min. The supernatant was then collected and then filtered using Wattman filter paper (5891 blackribbon, ashless) before passing through the rotary evaporator (Buchi Switzerland) at 40 °C. The solution obtained was introduced in an oven at 40 °C. The powder obtained was weighed and kept at + 4 °C protected from light [16]. 50 g of *Portulaca oleracea* powder was macerated in 500 mL of distilled water for 48 h at room temperature. During maceration, the whole macerate was, from time to time, agitated with a magnetic stirrer. This mixture was then centrifuged in a Centrifuger at 3500 rpm for 10 min. The supernatant was collected and then filtered using the filter paper. The filtrate was put directly into the oven at 40 °C, for complete evaporation [16].

Screening of the parts of Portulaca oleracea

The screening of the different parts of *Portulaca oleracea* (roots, leaves, stems and the whole plant) were performed on adult female *Haemonchus contortus* at concentrations of 1, 2 and 3 mg/mL each to determine the most effective part. Following the protocol described [19], adult female worms were incubated in 24-well plates, one worm per well containing 500 µL of solution of the different extracts, incubated at 37 °C in an incubator for 24 h, i.e. 6 worms per concentration. Observations were made under the microscope every 6 h for 24 h. Three tests were performed for each concentration.

Recovery of Haemonchus contortus eggs

Adult gravid female *Haemonchus contortus* worms were identified under a binocular lens, isolated, and placed in a petri dish containing PBS. These isolated worms were then placed in 24-well plates with 5 worms per well in 0.5 mL of PBS at 27 °C in the oven. Six hours later, a large quantity of eggs was deposited at the bottom of the well. After checking the presence of eggs in the medium with a binocular magnifying glass, these female worms were removed from the PBS and the contents were pipetted to concentrate them in a 50 mL Eppendorf tube. Centrifugation at 1000 rpm for 5 minutes was done. The supernatant was removed and the pellet containing the eggs was saved for testing.

Egg Hatch Inhibition Test

The *in vitro Haemonchus contortus* egg hatching test inhibition was based on the method described [17]. *Haemonchus contortus* eggs were incubated to evaluate the ovicidal activity of the aqueous and hydromethanolic extract of *Portulaca oleracea*. Thirty eggs of *Haemonchus contortus* were evaluated in a volume of 15 µL, deposited in the wells of the 24 plates and incubated at different concentrations: 5000, 4500, 4000, 3500, 3000 µg/mL of aqueous and hydromethanolic extract of *Portulaca oleracea*. Levamisole was used as a positive control and evaluated at the same concentration with PBS being used as a negative control. Each test was repeated 3 times for each extract. The plates were then covered and placed at 27 °C for 48 hours after which three drops of formalin were placed in each well to stop egg development. Then, the hatched eggs were counted under the microscope. After incubation, hatched and unhatched eggs were counted using a

binocular microscope at a magnification of (40 X). The percentage of egg hatch inhibition (% EHI) was calculated using the formula:

$$\% EHI = 100 - (\text{Number of } L_1 \text{ larvae} / \text{Number of fresh eggs in culture}) \times 100)$$

Recovery of infesting larvae

Infesting larvae were obtained by stool culture from goat faeces previously infested artificially with *Haemonchus contortus* L_3 and left in culture at 27 °C for 10 days [18]. They were extracted from the goat fecal mass by the Baermann device whose principle is based on the hygrotropism of L_3.

Larvicidal activity Test of Portulaca oleracea

Thirty L_3 of *Haemonchus contortus* were pipetted under a binocular magnifying glass (40×) using a 100 µL micropipette placed in each well of 24 and incubated with different concentrations (5000, 4500, 4000, 3500, 3000 µg/mL) of aqueous and hydromethanolic extract of *Portulaca oleracea* levamisol was used as positive control at the concentrations of 150; 300; 620; 1250 and 2500 µg/mL whereas PBS was used as negative control. Plates were then covered and placed at 27 °C for 24 and 48 h. After the incubation time, 10 µL of an enzymatic dye: MTT at 100 % was added to the larvae for 3 hours. The advantage of this step is that the MTT will cause enzymatic reactions which stain the living larvae blue-violet. Dead larvae will remain colorless. The count of paralyzed larvae appearing coiled up was carried without difficulty. The percent mortality of the larvae was determined using the following formula:
Mt (%) = (Number of dead larvae / Number of larvae in culture) ×100

Collection of adult Haemonchus contortus worms

Samples of goat/sheep abomasum curds with worms were collected at the Bantaille market in the city of Ngaoundere(Cameroon) and brought to the Applied Zoology Laboratory of the University Ngoundere. The worms were examined under a binocular microscope for identification of adult females of *Haemonchus contortus*.

Anthelmintic activity of Portulaca oleracea

Anthelmintic tests of aqueous and hydromethanolic extracts of *Portulaca oleracea* were performed following the protocol of [19]. Adult female worms were incubated in 24-well plates, one worm per well and 6 worms per concentration. The different concentrations (100, 300, 500, 700 and 1000 µg/ml) were administered. Levamisole and PBS were used as positive and negative controls, respectively. Three trials were performed for each concentration. The worms were incubated at 37 °C in an incubator and the mortality rate of the worms was assessed after 24 h by microscopic observation (20 ×) [16]. The percentage of survival was calculated by the formula:

Percentage survival = (Number of live worms) / (Total number of worms) × 100

Qualitative phytochemical test

Alkaloids were determined by the method described [20]. The flavonoids, leucoanthocyanine and terpenoids were obtained by the method described [21]. The tannins, the phenolic compounds were determined respectively by the methods of [22] et [23]. Les iridoids were detected by the method described [24].

Quantitative phytochemical test

Phenolic compounds were determined following the Folin-Ciocalteu (FC) method used [25]. The amount of condensed tannins was determined following the method described [26] in the presence of vanillic acid. The determination of total flavonoids was performed by the aluminum trichloride method [27]. For each test performed the trials were repeated three times.

Acute toxicity study of the aqueous extract of Portulaca oleracea on white mice

Animals

The mice and white rats were bought at LANAVET of Garoua (Cameroon) then, were acclimatized at 27 °C during 5 days in the laboratory of Applied Zoology of the University of Ngaoundere(Cameroon). The acute toxicity study was done according to the "dose adjustment" method of OECD guideline 425 [28]. Six white female mice were used to test the aqueous extract of the whole plant of *Portulaca oleracea* at the single dose of 2000 mg/kg body weight (bw). The test was performed on 6 nulliparous female mice, their behavior and the number of deaths were observed over a period of 14 days [29].

Study of the subacute toxicity of the aqueous extract of Portulaca oleracea on rats

To evaluate the subacute toxicity of the aqueous extracts of *Portulaca oleracea*, the "dose adjustment" method of the OECD guideline 407 [28] was used. The manipulations were done on white albino wistard rats of both male and female sexes. Their ages were between 8 and 12 weeks with an average weight between 85 and 144 g. The male and female rats, previously distributed, were placed in plastic basins numbered from 1 to 4 containing wood chips renewed at least twice a week. After acclimatization, 24 rats divided into 4 batches of 6, including 3 males and 3 females were fasted for 24 hours before administration of the extract. Throughout the experiment, the rats were maintained at 27 °C and subjected to an alternation of 12 h of light and 12 h of darkness in order to maintain their normal cycle. They were arranged as follows: batch 1, 2 and 3 receiving a solution of the extract at 200, 400 and 800 mg/kg body weight respectively and batch 4 (positive control) receiving distilled water at 1 mL/100 g body weight. Administration was spread over a 28-day period. The rats were fed daily and water consumption was continuous. They were weighed every 2 days from D_0 to D_{28} to allow us to calculate the percentage of weight gain (GP) by the formula below:

$$\% GP = (Mn - M(n-1))/M(n-1) \times 100$$

With, Mn: Average in day n; Mn-1: Average in day n-1; n: Number of days 0, 2, 4,.....28.

The extract gavage was performed daily between 9:00 and 10:00 am. At day 28 all 24 rats were sacrificed to perform hematological, biochemical, and histological analyses.

Choice of the administer doses

Following the acute toxicity tests carried out at a single dose of 2000 mg/kg bw on white mice *Mus musculus* and the observations made on them in addition to the literature review, the doses of 200, 400 and 800 mg/kg bw were assessed. Indeed, after administration

of the single dose 2000 mg/kg bw on the mice, they showed signs such as tremor, drowsiness, grooming. Considering the reaction of the mice after administration of 2000 mg/kg bw, the dose was reduced to 800, 400 and 200, mg/kg bw. Moreover, it is a repeated dose of 28 days so, if there is any abnormality, we will have the idea on the toxicity of the plant in the long term because *Portulaca oleracea* is also consumed by human beings.

Stages of collection of the organs of the rats

To collect the organs (heart, lung, liver, spleen, and kidneys), the rats were put in turn in dorsal decubitus then, the ventral face was incised in order to better locate these organs.
Each organ was taken and weighed on an electric scale in order to calculate the relative weights of the organs by the formula below:
Pr = Po / Pa × 100
Where, Pr: relative organ weight in g/100g; Po: organ weight in g; Pa: rat body weight in g.

After each weighing, liver, kidney, lung, spleen and heart were fixed by batch in each vial containing formalin previously concentrated to 40 % and then diluted to 10 % concentration (25 mL). It should also be noted that after each removal of these organs, a physiological liquid consisting of 0.9 % concentrated NaCl was constantly sprayed on the organs with the squeeze bottle so that they would not dry out. When the organs were collected after the animals were killed on the 28th day, the different parameters such as haematological and biochemical parameters were measured at the Medical Analysis Laboratory of the Vina Regional Hospital (Ngaoundere-Cameroon) and the histological parameters at the Animal Physiology Laboratory of the University of Yaoundé I.

Determination of haematological parameters

Hematological analysis was performed using an automatic blood count analyzer of Diatron group brand according to the method described by [30]. The whole blood of the animals treated with the aqueous extract of the whole plant of *Portulaca ortulaca oleracea* were collected in the EDTA (Ethylene Diamine Tetra-Acetic) tube. In this assay the parameters listed were: white blood cells (WBC), lymphocytes (LYM), neutrophils (MID), granulocytes (GRA); red blood cells (RBC), hemoglobin (HB), hematocrit (HT), mean corpuscular volume (MCV), mean cellular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCHC), platelets (PLT), thrombocrit (THT) and mean platelet volume (MPV) were determined according to [31].

Determination of biochemical parameters

The blood samples were collected from the rats by puncture at the retro-orbital sinus. The blood sample was collected in three different tubes. The EDTA tubes were centrifuged at 4000 rpm for 10 min and the serum obtained was stored at -20 °C for blood biochemical analysis [32].
The parameters involved were creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), urea and glucose.

Study of histological sections

The histological section of (liver, lungs and kidneys) was carried out in the laboratory of Animal Physiology of the University of Yaoundé I according to the protocol described [33].

Data analysis

Means, standard deviations were calculated using Microsoft Office 2016 software, determination of LC_{50} by the log-probit method using SPSS 16.0 software, and the Dunnett test was used for means comparisons. The difference was significant if $p < 0.05$.

Results

Screening of parts of Portulaca oleracea

Table 1 presents the results of the screening on different parts of *Portulaca oleracea*. From this table 1, it appears that the whole plant had more anthelminthic effects than the other parts with a proportion of 100% and 94.44 ± 7.85% of mortality respectively with the hydromethanolic extract of the whole part and the aqueous extract of the whole part of *Portulaca oleracea* at the concentration of 1 mg/mL after 24 h of incubation.

Ovicidal activity of Portulaca oleracea

Figure 1 represents the hatching inhibition rate of *Haemonchus contortus* versus the concentration of EA, EM, and levamisole. The egg hatching inhibition percentage ranged from 28.88 ± 9.62 % to 74.44 ± 1.92 % and 39.99 ± 6.66 % to 86.66 ± 8.21 % for *Portulaca oleracea* EA and EM, respectively, compared to Levamisole which ranged from 79.99 ± 3.33 % to 100 % at 48 h, for concentrations ranging from 3000 µg/mL to 5000 µg/mL.

Test on L_3 of Haemonchus contortus

Figure 2 shows us the percentage mortality of *Haemonchus contortus* L_3 at 48 h because of EA and EM of *Portulaca oleracea* whole plant as a function of concentration. At 48 h, the percentage of mortality for EA varied from 34.44 ± 3.84 % to 76.66 ± 3.33 % and for EM the variation was 31.10 ± 6.93 % to 88.88 ± 1.92 % versus that of levamisole which varied from 68.88 ± 7.69 to 100 %.

Female adult test of Haemonchus contortus

Figures 3 and 4 present the curve of the mortality rate of the adult mortality rate of *Haemonchus contortus* to EA and ME of *Portulaca oleracea* as a function of the concentrates. The mortality percentage of *Haemonchus contortus* ranged from 72.21 ± 9.62 % to 100 % and 55.55 ± 9.61 % to 100 % for EA and EM of *Portulaca oleracea* respectively versus that of levamisole which is 72.21 ± 9.62 to 100 % at 24 h (Figure 5).

Qualitative phytochemical tests

The phytochemical analysis revealed the presence of secondary metabolites namely phenols, flavonoids, saponins, tannins, glycerols and terpenoids in the aqueous extract and the presence of flavonoids, saponins, alkaloids, glycosides and terpenoids in the hydromethanolic extract of *Portulaca oleracea* (Table 2).

Quantitative phytochemical tests

The quantities of total polyphenols, tannins and flavonoids were determined from the linear equation regression line ($y = a x + b$) and expressed respectively in mg gallic acid equivalent, mg

catechin equivalent and mg rutin equivalent per 100 g of dry matter (Table 3)
EAG/g DM and 5.688 ± 0.309 mg RE/g DM compared to EA which has 12.998 ± 0.287 mg EAG/g DM and 1.847 ± 0.108 mg EC/g DM respectively. On the other hand, we have more tannins in EA with 5.688 ± 0.309 mg RE/g DM, compared to ME which is 1.847 ± 0.108 mg EC/g DM.

Toxicity Test

Acute toxicity test

After 14 days of observation, no deaths were recorded in treated female mice. After oral administration of 2000 mg/mL bw of the *Portulaca oleracea* AE, the mice showed signs of tremor for 2 hours times and late drowsiness for 3 hours, but no mortality case. According to the [34]. Toxicity scale, the toxicity dose is between 2000 and 5000 mg/kg bw from which, we can say our plant is moderately toxic.

Test of subacute toxicity

Figures 6 and 7 show the effect of subacute administration of aqueous extract of *Portulaca oleracea* whole plant on the weight of female rats. It can be seen from Figure 6 that the change in weight of the rats is increasing until D_{28}. However, we did not find a significant difference between 200, 400 and 800 mg/kg bw dose compared with the control ($P > 0.05$). Figure 7 shows female rats at 400 mg/kg bw, we observe that there was a weight loss at D_2 and then an increase in weight up to D_{28}. There was no significant difference from D_0 to D_{16} at 200 mg/kg bw ($P > 0.05$), but from D_{16} to D_{28} there was a highly significant difference at the same dose compared to the control ($P < 0.001$). However, there was no significant difference ($P > 0.05$) at 400 and 800 mg/kg bw compared to the control. In general, up to the 28th day we did not record a statistically significant difference on the evolution of the weight of the animals in both males and females. Table 4 shows the variation in organ weights of male and female rats after necropsy due to the administration of the aqueous extract of the whole plant of *Portulaca oleracea* at different doses compared to the control. After analyzing this Table 4, we found that $P > 0.05$ between all organs collected (liver, lung, heart, kidney, and spleen) at 200, 400 and 800 mg/kg bw and the control organs in males. In contrast, a moderately significant difference ($P < 0.05$) is reported in the same Table 4 in the liver of female rats at 200 mg/kg bw due to the aqueous extract of *Portulaca oleracea* compared to the control.
Table 5 shows the results of the biochemical parameter of male and female rats due to the aqueous extract of the whole plant of *Portulaca oleracea* administered at doses of 200, 400 and 800 mg/kg bw. It shows from this Table 5 that there is no significant difference ($P > 0.05$) between creatinine, urea, ALT, ASAT and glucose compared to the control at 200 and 800 mg/kg of bw in males. Except the significant difference ($P < 0.01$) reported at 400 mg/kg bw compared to the control but which is no longer at the 800 mg/kg bw dose.

From the same Table 5, it is evident that no significant difference ($P > 0.05$) was reported between creatine, urea, ALAT, ASAT and glucose compared to the control at 200, 400 and 800 mg/kg bw in female rats. Table 6 shows the variation of hematological parameters like white blood cells, red blood cells and platelets of male and female rats due to the administration of the aqueous extract of the whole plant of *Portulaca oleracea* compared to the controls. From this Table 6, there was no significant difference ($P > 0.05$) between Neutrophils and Granulocytes compared to the control at 200, 400 and 800 mg/kg bw. On the other hand, a highly significant difference ($P < 0.001$) was noted between the white blood cells, and the control at 200, 400 and 800 mg/kg bw and the lymphocytes at 200 mg/kg bw. We also observed a moderately significant difference ($P < 0.05$) between lymphocytes and the control at doses 400 and 800 mg/kg bw in male rats.
From the same Table 6, there is a highly significant difference ($P < 0.001$) between white blood cells and lymphocytes at 200, 400 and 800 mg/kg bw compared to the control in the female's rats. However, a significant difference was not recorded ($P > 0.05$) between neutrophils and granulocytes at the three doses compared to the control. In the same Table 6, we see that there is no significant difference ($P > 0.05$) recorded between red blood cells, hemoglobin, HT, MGV, MCC, MCCT compared to the control at doses 200,400 and 800 mg/kg bw following the administration of aqueous extract of *Portulaca oleracea* in male rats as well as in female rats. A highly significant difference ($P < 0.001$) between platelets and control at 200, 400 and 800 mg/kg bw was observed. In contrast we did not record a significant difference ($P > 0.05$) between THT and VPM at the same doses compared with the control of the two sexes.

Histological section

The histological section of the organs (liver, kidney, and lung) of male and female rats having undergone the administration of the aqueous extract of the whole plant of *Portulaca oleracea* showed a total architecture of these organs. After observing these three organs compared to the controls, we noted that there was no significant difference between them. A morphological view of these sections showed an identical architecture in kidney, liver and lung compared to the control for all doses administered at 200, 400 and 800 mg/kg bw for this plant. Figures 8 and 9 represent microphotographs of histological sections of rat liver, kidney and lung stained with hematoxylin-eosin. Hematoxylin stains the nucleus purple while eosin stains the cytoplasm pink.

Discussion

From figure 1, EA and EM from the whole plant of *Portulaca oleracea* inhibited a large number of *Haemonchus concortus* eggs in a concentration-dependent manner compared to the positive control. We found that there was no significant difference ($P > 0.05$) between EA and ME at 48 h of incubation with LC_{50} of 3.65 ± 0.16 for EA and 3.44 ± 0.13 for ME. But a highly significant difference ($P < 0.001$) was reported between EA and EM of *Portulaca oleracea* and levamisole at 48 h of incubation on *Haemonchus contortus* eggs and, as the concentration increased the rate of inhibition of egg hatching increased.

This result corroborates those of Yongwa et al. [35] who obtained ovicidal activity with the aqueous and ethanolic extract of *Senna italica* on *Haemonchus contortus* eggs. The concentrations used by the different authors differ from one plant to another. This is the case of the work of Assis et al. [36] who worked on the extracts of *Spigelia anthelmia*: an effectiveness of the inhibition of the eggs hatching concentration of 50 mg/mL was noted. Several authors have provided evidence that the high efficacy of plants may be due to secondary metabolites (condensed tannins, saponins, and flavonoids) included in the plant material, which are gradually released into the rumen of animals. We can also say that

Portulaca oleracea, has the capacity to act on *Haemonchus contortus* eggs, since some secondary metabolites are observed in EA and EM. These secondary metabolites act by penetrating the eggshell, the cuticle of the larvae by osmosis through the circulatory system and the process stops at the development of the blastomere of the larvae and this causes the death by starvation [37] or by paralysis [38].

Figure 2 shows the mortality percentage of *aemonchus. contortus* L_3 compared to the concentration of EA and EM of the whole plant of *Portulaca oleracea*. From this table, it appears that EA and EM of *Portulaca oleracea* showed efficacy on *Haemonchus contortus* eggs L_3 in a progressive manner. After 48 h of incubation there was no significant difference ($P > 0.05$) between the EA and EM. On the other hand, a highly significant difference ($P < 0.001$) was reported between levamisole versus EA and EM of *Portulaca oleracea*. The percentage of mortality varied from 34.44 ± 3.84 % to 76.66 ± 3.33 %, with an LC_{50} of 3.61 ± 0.14 and from 31.10 ± 6.93 % to 88.88 ± 1.92 %, with an LC_{50} of 3.54 ± 0.10 respectively for EA and ME of *Portulaca oleracea* compared to levamisole which percentage of mortality varies from 68.88 ± 7.69 % to 100 %, with an LC_{50} of 2.76 ± 0.15. As with the *Haemonchus contortus* egg hatching inhibition test, we recorded a significant larvicidal activity of our plant extracts on *Haemonchus contortus* stage 3 larvae. These results are similar to those obtained by some authors such as Brunet et al. [39] who showed that sainfoin extract, a plant rich in tannins, affects the unsheathing kinetics of *Haemonchus contortus* L_3 and that this inhibitory effect depends on the extract concentration. Similarly, [40] had shown that some plants rich in tannins could partially or completely inhibit the *in vitro* migration of L_3 larvae. From the results of these authors compared to ours, we found that EA and EM from the whole plant of *Portulaca oleracea* had a mortality rate of 76.66 ± 3.33 % and 88.88 ± 1.92 respectively at the concentration of 5 mg/mL. We concluded that although our plant extracts had larvicidal effects on *Haemonchus contortus* L_3, these L_3 are very resistant hence the use of high dose concentrations of our plant extracts. According to the method developed by Ndjonka et al. [16], the death of L_3 is confirmed by the change of coloration of the larvae after 48 h of incubation for 30 min and we observe the change of the colors of the larvae: purple for the living L_3 and yellow for the dead L_3.

The *in vitro* tests of the aqueous and hydromethanolic extracts of the whole plant of *Portulaca oleracea* acted significantly on the adult female worms of *Haemonchus contortus*, gastrointestinal parasite of small ruminants. The percentage of worm mortality ranged from 72.21 ± 9.62 % to 100 % with an LC_{50} of 0.057 ± 0.022 and 55.55 ± 9.61 % to 100 % with an LC_{50} of 0.096 ± 0.029 respectively for EA and EM of *Portulaca oleracea* compared to that of levamisole which is 72.21 ± 9.62 % at 100 % with an LC_{50} of 0.069 ± 0.005 at 24 h incubation at concentrations ranging from 100 to 1000 µg/mL. These results are almost like those obtained by Akkari et al. [41] who recorded 91.3 % and 100 % mortality at 2 mg/mL, concentration after 8 and 24 h of exposure to crude ethanolic extract of *Artemisia campestris*. However, our results differ from those obtained by Yongwa et al. [35] who worked on the *in vitro* anthelmintic activity of the aqueous and ethanolic extract of *Senna italica* on three stages of *Haemonchus contortus*. Several authors have demonstrated that anthelmintic activities in plants are related to the amount of condensed tannins and/or other secondary metabolites such as flavonoids, steroids, terpenoids, alkaloids, saponins, etc [42 - 43]. The present study shows that the aqueous and hydromethanolic extract of the whole plant of *Portulaca oleracea* had an anthelmintic effect on adult female worms of *Haemonchus contortus*.

The use of *Portulaca oleracea* by farmers for the treatment of small ruminants could be explained by the presence of secondary metabolites having pharmacological effects on gastrointestinal nematodes. Among others were noted the presence of saponins presented by many authors as molecules suspected of having anti-helminthic properties [44]. Tannins have also been listed as having anthelmintic properties against *Haemonchus contortus* [41]. From all these pharmacological properties of *Portulaca oleracea*, it is believed that it is through these bioactive metabolics that this plant acts on nematodes in general and *Haemonchus contortus*.

The contents of secondary metabolites such as the total polyphenols, tannins and flavonoids were determined. We found that the content of the hydromethanolic extract is more consistent in phenolic compounds and flavonoids compared to the aqueous extract. On the other hand, the tannin content is less representative in the aqueous extract compared to the hydromethanolic extract. These variations in content may be due to several factors such as: climate, the stage of development of the plant and its degree of ripening, the time of harvest, the duration of storage, the method of extraction and the method of quantification of compounds of biological interest [45]. Studies have shown that polyphenols, saponins and tannins possess anthelmintic activities [46]. These chemical families were listed in the aqueous and hydromethanoic extract of *Portulaca oleracea* and are believed to be the source of ovicidal and larvicidal activity of *Haemonchus contortus*.

To minimize as much as possible, the risks of toxicity of our plants and to have an improved drug of good quality and without toxic effect, it was important for us to deepen the research on the degree of toxicity of *Portulaca oleracea*. Thus, the study of acute toxicity by oral route of the aqueous extract of the whole plant of *Portulaca oleracea* allowed us to continue our tests on the subacute toxicity in order to highlight the histological sections of the liver, kidneys and lung. We chose the oral route because it is the usual route involved under normal conditions for humans and ruminants. We did not record a statistically significant difference between the aqueous and hydromethanolic extract of the whole plant of *Portulaca oleracea* on *Haemonchus contortus*. However, we preferred to use the aqueous extract as the tradithérapeute's use. The results obtained with these plant extracts showed no signs of toxicity in mice for 14 days. The aqueous extract of *Portulaca oleracea* administered orally at a single dose of 2000 mg/kg bw revealed an LD_{50} greater than 5000 mg/kg bw and did not cause any deaths throughout our study. According to the OECD Globally Harmonized Classification System [47], our total aqueous extract can be classified at the 5^{th} category and considered as a non-toxic substance by the oral route. The LD_{50} was obtained by the limit "dose adjustment" method of OECD guideline 425 [28]. For the continuity of our tests, the doses of the aqueous extract of the whole plant of *Portulaca oleracea* used are doses strictly below to 2000 mg / kg bw. Drowsiness, grooming, and trembling were observed at the 4 h after administration of *Portulaca oleracea* extract until day 14. This result is in line with that obtained by Etamé et al. [29] who worked on the wine extract of *Carica papaya* Linn seeds at the same dose and obtained no deaths of mice until D_{14}. *Portulaca oleracea* averagely is nontoxic because we did not have the case of mortality until D_{14} at the same dose of 2000 mg/kg bw. In order to evaluate the long-term toxicity of *Portulaca oleracea*, it is important to evaluate the subacute toxicity of the latter.

The subacute toxicity tests of these plant extracts revealed encouraging results because the administration of the aqueous extract of the whole plant of *Portulaca oleracea* was

spread over a period of 28 days. The organs were weighed and observed physically and morphologically under the microscope to see if they were affected by the extracts of our plant or not. During the continuous oral administration of our plant extract, weights were regularly raised after two successive days in male and female rats. The loss of weight is correlated with the physiological state of the animal and can be explained, not only by anorexia, but also by the alteration of the metabolism of the animals as reported [48]. In our case, the variation in weight of the animals due to the aqueous extract of the whole plant of *Portulaca oleracea* revealed that there was no statistically significant difference ($P > 0.05$) observed on the evolution of the weight of the animals in both males and females over a period of 28 days. Regarding the morphological analysis of the organs of the rats (males and females) we found that there was no significant difference ($P > 0.05$) between these organs (liver, kidneys, spleen, lung, and heart) and the control. However, a moderately significant difference ($P < 0.05$) in the liver of female rats at 200 mg/kg body weight due to the aqueous extract of the whole plant of *Portulaca oleracea* compared to the control was reported. Generally, the change in internal organ weights is an indication of toxicity after exposure to a toxicant [49]. This is not the case with our plant extract because the organs of our animals did not change in weight compared to the control.

The liver, kidneys and lung, organs that usually regulate metabolism and excretion, are particularly sensitive to potential toxic agents: their function must therefore be monitored in toxicological studies [50]. Statistical analysis performed showed stable AST and ALT levels in both sexes at all doses. That transaminases or aminotransferases are tissue enzymes that catalyze the transport of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid. Transaminases are present in the liver, but also in muscle, kidney, pancreas, and other tissues. They are synthesized in the cytoplasm of cells in these organs and discharged into the circulation when these cells are damaged [51]. Still called leukocytes, white blood cells play a very important role in the protection and defense of the body against bacteria, foreign substances, viruses, parasites, toxins, and tumor cells [52]. When its value is higher than normal, it can indicate an activation of the immune system in response to an infection, inflammation, necrosis, or malignant disease [52]. We also recorded an elevated white blood cells count in both male and female rats due to the administration of the aqueous extract of the whole plant of *Portulaca oleracea* compared to the controls. The primary function of red blood cells is to transport oxygen from the lungs to the tissues [53]. When comparing the red blood cell levels in the test set to the control, we found that there was no variation in values for both male and female rats of the aqueous extract of the whole plant of *Portulaca. oleracea*. We recorded a highly significant difference ($P < 0.001$) between the AE of the whole plant of *Portulaca oleracea* on the platelets at doses 200, 400, and 800 mg/kg bw compared to the input of the controls in both male and female rats. This increase in the platelet count can lead to thrombocytosis, which could be the cause of vascular, venous, or arterial obstruction.

Figures 8 and 9 represent histological sections of the kidneys at objective (×200), liver at objective (×100), and lungs at objective (×40) of male and female rats, respectively, that were administered with the aqueous extract of the whole plant of *Portulaca oleracea*. The letters A, B, and C in Figures 9 and 10 represent the batches receiving the extract of these plants at the doses of 200, 400, and 800 mg/kg bw, respectively, and the letter D represents the normal control batch of all the highlighted organs.

However, many plant-based compounds accumulate after their absorption in some organs, especially the liver and kidneys, responsible for biotransformation and excretion [54], The architecture of the liver D_1 marked by the negative control batch for all figures, reveals to us a normal aspect without hepatic modifications, the hepatocytes which, are large polyhedral cells with large round nuclei with the nucleolus well visible, are organized in a radial way around the centrilobular veins which receive blood from the hepatic parenchyma in contact with the sinusoidal capillaries. In addition, the D_2 kidneys are major targets of induced toxicity by toxic substances and their derivatives because of its functions of filtration, urinary evacuation and elimination of chemical derivatives that result from different metabolic pathways. Exposure of the kidneys to these substances can cause an alteration in the globular and tubular structure [55]. This toxicity is determined by analysis of renal biochemical factors such as urea and creatinine. In our work for these two renal factors (urea and creatinine) a significant difference between the two was not highlighted. However, the architecture of the kidneys shows the normal aspect of the organ with the different visible parts such as the glomeruli which are surrounded by a capsule and ensure the glomerular filtration of blood and the passage of water and low molecular weight compounds into the urinary tract. We also observe the urinary space or glomerular chamber that surrounds the glomeruli, the distal and proximal convoluted tubules on either side of the glomerulus. The lung, on the other hand, represents an organ that is very sensitive to direct aggression from the external environment. In the organism, it has a unique situation because it has an extremely developed vascular network [56]. By submitting the lung to the histological section, with the objective (x 40), we manage to highlight the visible parts such as the pulmonary bronchiole, the pulmonary arteriole, and the alveolar sac. The architecture of the animal's lung after the histological montage shows that all the parts are normally represented. From the results obtained on the different organs (liver, kidneys, and lung) concerning the histological sections, we conclude that none of these organs have been affected hence the *Portulaca oleracea* could be used for the manufacture of an improved drug because they were no toxic effect and could be consumed as much as possible by humans and animals (ruminants).

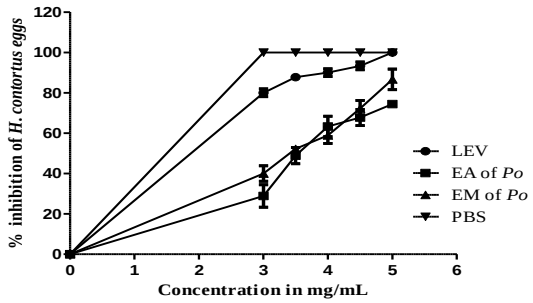

Figure 1. inhibition variation mortality curve rate of *Haemonchus contortus* eggs due to EA and ME of *Portulaca oleracea* levamisole as a function of the concentration

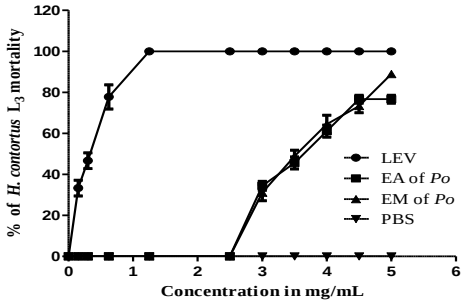

Figure 2. variation mortality rate of L_3 of *Haemonchus contortus* due to the effect of AE and ME of *Portulaca oleracea* and levamisole at 48 h compared to the concentration

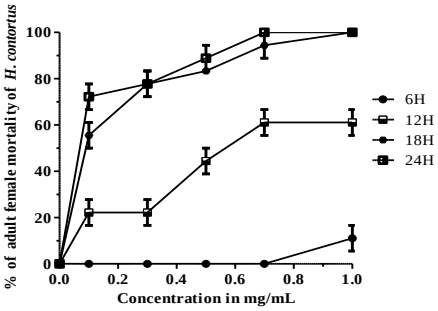

Figure 3. variation mortality rate of *Haemonchus contortus* worms due to *Portulaca oleracea* AE as a function of concentrations

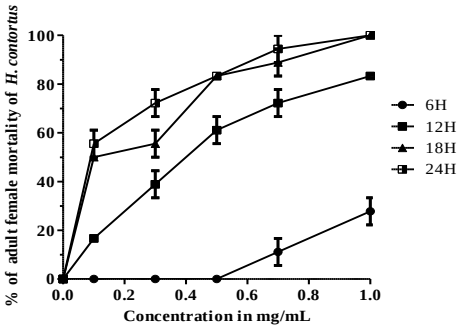

Figure 4. variation mortality rate of *Haemonchus contortus* worms due to the ME of *Portulaca oleracea* as a function of concentration

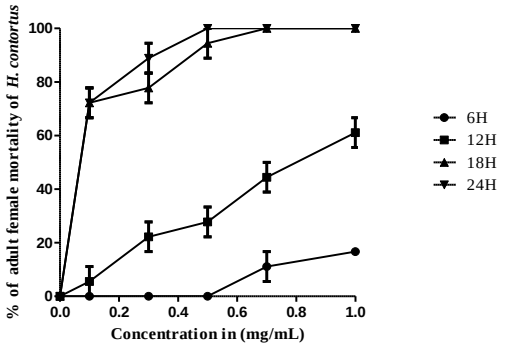

Figure 5. variation mortality rate of *Haemonchus contortus* worms in the positive control (Levamisole) as a function of concentration.

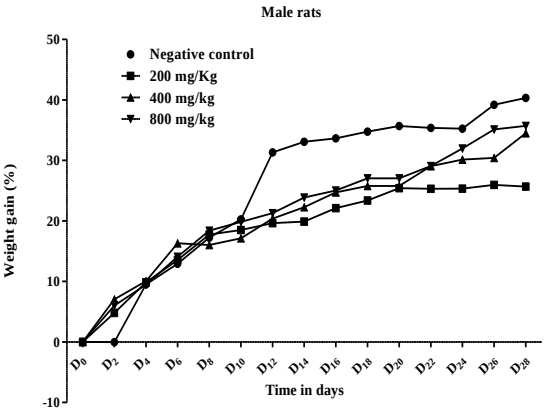

Figure 6. effect of subacute administration of aqueous extract of *Portulaca oleracea* whole plant on the weight of male rats.

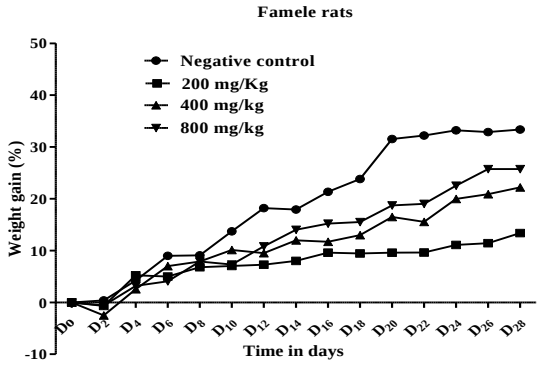

Figure 7. effect of subacute administration of aqueous extract of *Portulaca oleracea* whole plant on the weight of female rats

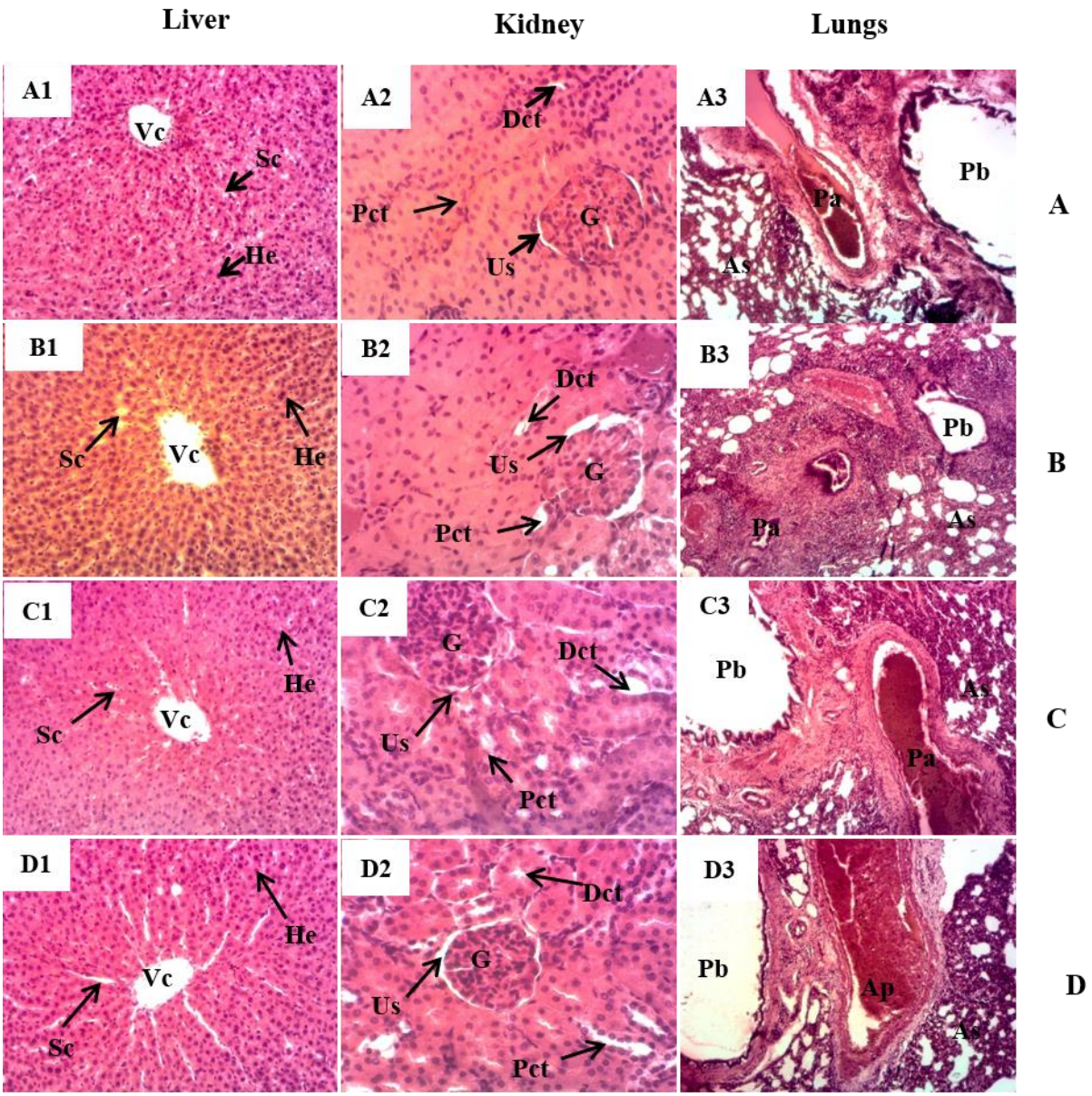

Figure 8. Microphotographs of liver (X100), kidney (X200) and lungs (X40) of male rats.
HE. A, B, C = Batches receiving *Portulaca oleracea* extract at different doses; D = Normal control; Liver; Vc = Centrilobular vein; He = Hepatocyte; Sc = Sinusoidal capillary; Kidney; G = Glomerulus; Us = Urinary space; Dct = Distal convoluted tubule; Pct = Proximal convoluted tubule; Lungs; Pb = Pulmonary bronchiole; Pa = Pulmonary arteriole; As = Alveolar sac

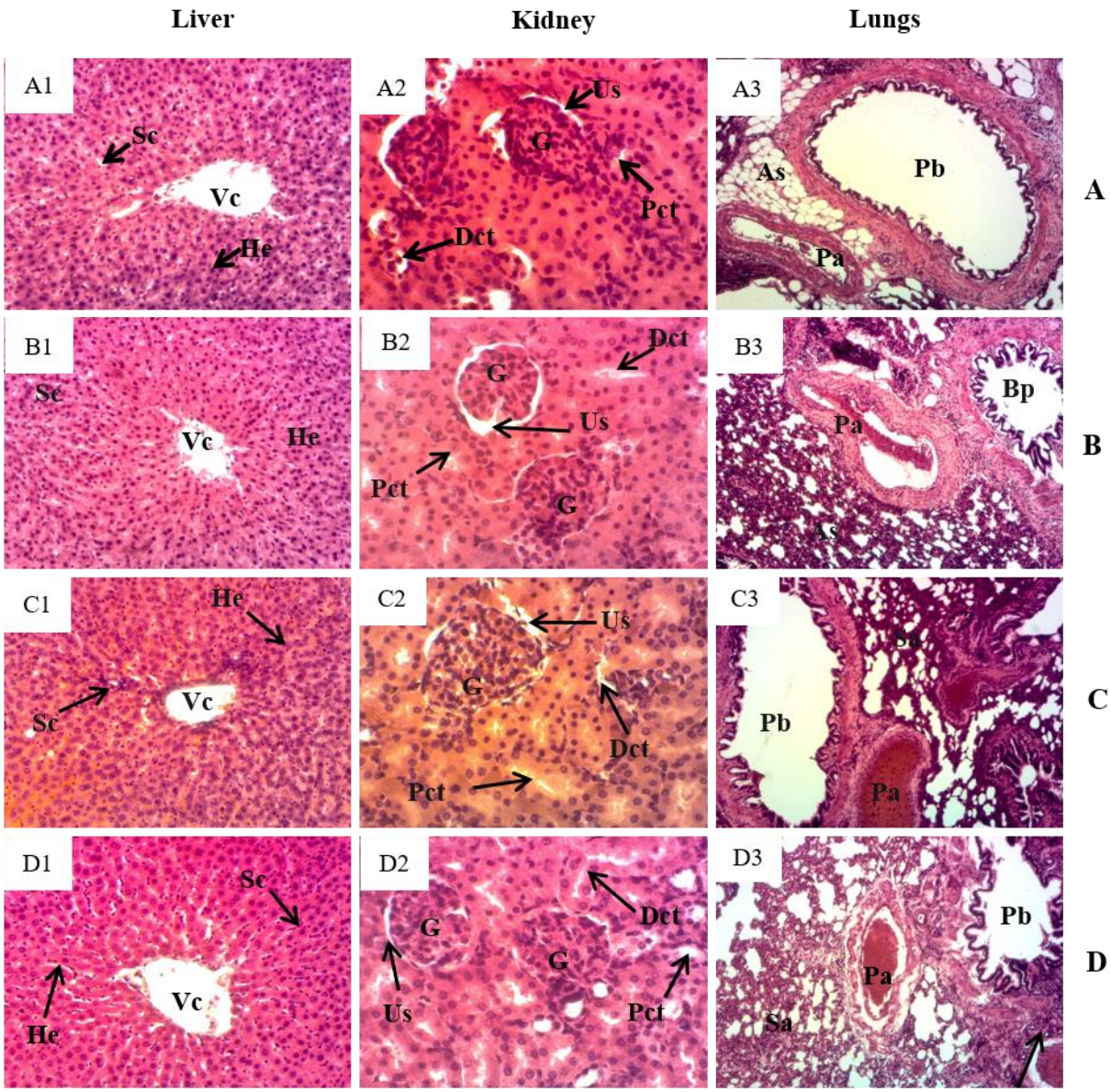

Figure 9. microphotographs of liver (X 100), kidney (X 200) and lungs (X 40) of female rats.

HE. A, B, C = Batches receiving *Portulaca oleracea* extract at different doses; D = Normal control; Liver; Vc = Centrilobular vein; He = Hepatocyte; Cs = Sinusoidal capillary; Kidney; G = Glomerulus; Eu = Urinary space; Tcd = Distal convoluted tubule; Tcp = Proximal convoluted tubule; Lungs; Br = Pulmonary bronchiole; Ap = Pulmonary arteriole; Sa = Alveolar sac.

Table 1. Screening of the different parts of *Portulaca oleracea*

Parts of the plant	Conc in mg/mL	Mortality rate in percent (%) Mean ± Standard deviation 24 hours		
		Aqueous extracts	hydromethanolic extracts	Hydroethanolic extracts
Roots	1	77.77 ± 15.71	88.88±15.71	66.66 ±13.6
	2	83.33 ± 13.61	88.88±15.71	83.33 ± 13.61
	3	88.88 ± 7.85	100	94.44 ± 7.85
Leaves	1	72.22 ±15.71	72.21 ± 7.85	88.87 ±7.85
	2	72.22 ± 15.71	100	88.87 ±7.85
	3	83.33 ± 13.61	100	100
Stems	1	61.1 ± 7.85	66.66 ±13.6	66.66 ±13.6
	2	72.22 ± 15.71	77.77 ± 15.71	88.88 ± 7.85
	3	83.33 ± 13.61	95.55 ± 6.28	94.44 ± 7.85
Whole plant	1	94.44 ± 7.85	100	83.33 ±13.61
	2	100	100	100
	3	100	100	100

Conc = Concentration

Table 2. Qualitative phytochemical tests result of *Portulaca oleracea*

Secondary metabolites	***Portulaca oleracea* Extracts**	
	Aqueous	**Hydromethanolic**
Phenols	+	-
Flavonoids	+	+
Saponins	+	+
Alkaloids	-	+
Tannins	+	-
Glycosids	+	+
Terpenoids	+	+
Iridoids	-	-

(+): Present; (-): Absent

Table 3. Quantitative phytochemical tests of EA and EM of the whole plant of *Portulaca. oleracea*

Plant	Extracts	**Total polyphenols**	**Tannins**	**Flavonoids**
		mg EAG/g DM	mg EC/g DM	mg RE/g DM
Portulaca oleracea	Aqueous	12.998 ± 0.287	5.036 ± 0.273	1.405 ± 0.054
	Hydromethanolic	50.884 ± 0.535	1.847 ± 0.108	5.688 ± 0.309

Table 4. variation in weights of male and female rats organs due to aqueous extract of the whole plant of *Portulaca oleracea* compare to administration time.

Organs	**Control**		**200 mg/kg**		**400 mg/kg**		**800 mg/kg**	
	males	female	males	female	males	female	males	female
Liver	3.75 ± 0.11	4.51 ± 0.46	3.31 ± 0.02 ^{ns}	3.79 ± 0.06**	3.49 ± 0.42 ^{ns}	4.14 ± 0.26 ^{ns}	3.45 ± 0.03 ^{ns}	4.08 ± 0.11 ^{ns}
Lung	0.88 ± 0.07	0.97 ± 0.06	1.03 ± 0.10 ^{ns}	0.93 ± 0.09 ^{ns}	0.95 ± 0.31 ^{ns}	1.17 ± 0.42 ^{ns}	0.87 ± 0.18 ^{ns}	1.24 ± 0.34 ^{ns}
Core	0.35 ± 0.02	0.41 ± 0.02	0.31 ± 0.01 ^{ns}	0.39 ± 0.00 ^{ns}	0.35 ± 0.01 ^{ns}	0.36 ± 0.00 ^{ns}	0.36 ± 0.03 ^{ns}	0.40 ± 0.03 ^{ns}
Kidneys	0.61 ± 0.04	0.77 ± 0.04	0.62 ± 0.01 ^{ns}	0.7 ± 0.01 ^{ns}	0.60 ± 0.03 ^{ns}	0.70 ± 0.01 ^{ns}	0.62 ± 0.02 ^{ns}	0.68 ± 0.06 ^{ns}
Rate	0.25 ± 0.02	0.59 ± 0.21	0.01 ± 0.04 ^{ns}	0.33 ± 0.02 ^{ns}	0.28 ± 0.14 ^{ns}	0.39 ± 0.06 ^{ns}	0.33 ± 0.03 ^{ns}	0.36 ± 0.04 ^{ns}

Highly significant difference (P < 0.001 (***)); Moderately significant (P < 0.05(**)); Significant difference (P < 0.01(*)); No significant difference (P > 0.05 (ns)).

Tableau 5. Variation in biochemical parameters of male and female rats as aqueous extract of the whole plant of *Portulaca oleracea* was administered with time.

Parameters	**Control**		**200 mg/kg**		**400 mg/kg**		**800 mg/kg**	
	males	**Female**	**males**	**female**	**males**	**female**	**males**	**female**
Creatine (mg/l)	0.73 ± 0.04	0.86 ± 0.16	0.7 ± 0.08 ^{ns}	0.76 ± 0.09 ^{ns}	0.73 ± 0.04 ^{ns}	0.8 ± 0.08 ^{ns}	0.73 ± 0.04 ^{ns}	0.76 ± 0.09 ^{ns}
ASAT (U/L)	242.66 ± 8.99	245.66 ± 39.38	215 ± 9.41 ^{ns}	196.66 ± 12.47 ^{ns}	282.33 ± 26.24*	281.66 ± 22.15 ^{ns}	256.66 ± 16.99 ^{ns}	225.00 ± 40.84 ^{ns}
ALAT (U/L)	105 ± 17.10	84 ± 4.96	86.66 ± 11.67 ^{ns}	68 ± 13.14 ^{ns}	118.66 ± 34.17 ^{ns}	93 ± 7.78 ^{ns}	139.33 ± 13.22 ^{ns}	67 ± 11.31 ^{ns}
Urea (mg/dL)	37.53 ± 6.40	31.7 ± 3.90	35.1 ± 1.55 ^{ns}	38.93 ± 4.13 ^{ns}	36.2 ± 6.93 ^{ns}	40.8 ± 0.98 ^{ns}	51.7 ± 7.53 ^{ns}	29.33 ± 3.45 ^{ns}
Glucose (mg/dL)	100.60 ± 5.55	123.62 ± 4.08	128.41 ± 10.43 ^{ns}	109.31 ± 5.74 ^{ns}	119.22 ± 10.11 ^{ns}	110.56 ± 2.92 ^{ns}	115.53 ± 11.50 ^{ns}	111.39 ± 8.47 ^{ns}

Highly significant difference (P < 0.001 (***)); Moderately significant (P < 0.05(**)); Significant difference (P < 0.01(*)); No significant difference (P > 0.05 (ns)).

Table 6. Male and female rats Hematological parameters due to the administration of the AE of *Portulaca oleracea*

Parameters	Control		200 mg/kg		400 mg/kg		800 mg/kg	
	males	female	males	female	males	female	males	female
WBC (x 10^9/L)	5.34 ± 1.06	12.22 ± 1.43	12.27 ± 0.02***	4.25 ± 0.15***	8.31 ± 1.99***	4.62 ± 0.36 ***	8.58 ± 1.83***	6.32 ± 0.93***
LYM (x 10^9/L)	3.62 ± 0.46	10.68 ± 1.19	8.54 ± 3.42***	3.24 ± 0.26***	5.45 ± 0.70**	3.12 ± 0.81***	6.52 ± 2.44**	4.18 ± 1.80***
MID (x10^9/L)	0.39 ± 0.20	0.64 ± 0.09	0.47 ± 0.27 ns	0.24 ± 0.06 ns	0.66 ± 0.32 ns	0.53 ± 0.28 ns	0.57 ± 0.23 ns	0.63 ± 0.30 ns
RBC (x10^9/L)	1.33 ± 1.20	0.89 ± 0.22	1.93 ± 1.05 ns	0.77 ± 0.35 ns	2.20 ± 1.00 ns	0.97 ± 0.24 ns	1.49 ± 0.40 ns	1.64 ± 0,67 ns
GR (x 10^{12} / L)	8.41 ± 0.29	7.20 ± 1.21	7.70 ± 1.07 ns	7.3 ± 0.88 ns	8.01 ± 0.96 ns	7.83 ± 0.71 ns	9.3 ± 1.03 ns	8.27 ± 0.29 ns
HB (g / dl)	13.6 ± 0.14	12.26 ± 1.71	13.36 ± 0.77 ns	12.36 ± 0.61 ns	12.86 ± 1.16 ns	12.86 ± 1.01 ns	14.6 ± 1.45 ns	13.43 ± 0.57 ns
HT (%)	42.86 ± 0.26	39.02 ± 3.65,	39.99 ± 3.49 ns	38.47 ± 3.97 ns	40.98 ± 3.41 ns	39.83 ± 3.58 ns	44.10 ± 1.87 ns	42 ± 0.75 ns
MGV (fl)	51 ± 1.63	51.66 ± 0.47	50.33 ± 0.47 ns	52.66 ± 1.24 ns	51.33 ± 2.05 ns	51 ± 0.81 ns	50.33 ± 0.47 ns	51 ± 0.81 ns
MCC (g/l)	31.8 ± 0.29	33.2 ± 1.06	32.03 ± 1.16 ns	32.4 ± 1.80 ns	31.4 ± 0.35 ns	32.3 ± 0.71 ns	31.3 ± 0.35 ns	31.93 ± 0.73 ns
MCCT (Pg)	16.2 ± 0.43	17.13 ± 0.74	16.1 ± 0.57 ns	17.1 ± 1.22 ns	16.13 ± 0.54 ns	16.43 ± 0.16 ns	15.66 ± 0.20 ns	16.23 ± 0.28 ns
PLT (x10^9/L)	325.66 ± 5.43	409.66 ± 13.91	673.33 ± 9.46***	450.33 ± 22.39***	476.66± 15.86***	535.66 ± 27.74***	457.33 ± 13.19***	341 ± 24.91***
THT (%)	0.3 ± 0.09	0.36 ± 0.07	0.44 ± 0.13 ns	0.26 ± 0.08 ns	0.44 ± 0.10 ns	0.37 ± 0.04 ns	0.27 ± 0.10 ns	0.28 ± 0.06 ns
MPV (fl)	6.96 ± 0.26	7.76 ± 0.74	7.96 ± 0.09 ns	7.06 ± 0.26 ns	7.6 ± 0.35 ns	7.36 ± 0.20 ns	7.43 ± 0.16 ns	7.1 ± 0.37 ns

Highly significant difference (P < 0.001 (***)); Moderately significant (P < 0.05(**)); Significant difference (P < 0.01(*)); No significant difference (P > 0.05 (ns)).WBC: White blood cells; LYM: Lymphocytes; MID: Neutrophils; GRA: Granulocytes; RBC: Red blood cells; HB: Hemoglobin, HT: Hematocrit; MGV: Mean corpuscular volume; MCC: Mean cell concentration of hemoglobin; MCCT: Mean corpuscular hemoglobin content; PLT: Platelets; THT: Thrombocrit; MPV: Mean platelet volume.

Conclusion

We had to evaluate the anthelmintic effect of the aqueous and hydromethanolic extract of the whole plant of *Portulaca oleracea* on eggs, stage 3 larvae and adult female worms of *H. contortus*, as well as the acute and subacute toxicity tests. Both aqueous and methanolic extracts showed encouraging results on all stages of the development of the parasite. The concentrations used on eggs and L_3 were higher than those on adults. We found that eggs and L_3 are more resistant than adults to the aqueous and hydromethanolic extracts of *Portulaca oleracea*. Of all the results obtained on the different stages of development of the parasite, Levamisole showed us a higher effect (P < 0.001) compared to both extracts of this plant but, a significant difference (P > 0.05) was not reported between the aqueous and hydromethanolic extract. Regarding the toxicity study performed on mice and rats, *Portulaca oleracea* is not toxic. The use of *Portulaca oleracea* is justified by breeders on ruminants in general and its use by traditional healers on humans. In order to know more about *Portulaca oleracea*, it will be important to carry out an *in vivo* toxicity study in small ruminants to see the effect of this plant on them.

Abbreviations

Abbreviations
AE: Aqueous Extract
ANOVA: Variance analysis
DMSO: Dimethyl Sulfoxide
IRAD: Instituate of Agriculture and Agronomic Research
LANAVET: National Veterinary Laboratory
LC: Lethal Concentration
ME: Hydromethanolic Extract
OECD: Organisation for Economic Co-operation and Development
PBS: Phosphate Buffer Solution
bw: Body weight
WHO: World Health Organization

Authors' Contribution

AA carried the field work. HI and NF carried laboratory experiments. AEI translated the manuscript from French language into English Language. VT and ND supervised the whole work.

Acknowledgments

The authors would like to thank the University of Ngaoundereand more specifically the Laboratory of Applied Zoology of the same institution for allowing us to carry out all this research work.

Conflict of interest

The authors declare no conflict of interest

Article history:
Received: 21 November 2021
Received in revised form: 16 December 2021
Accepted: 17 December 2021
Available online: 17 December 2021

References

1. National Institute of Statistic of Cameroon, 2015 edition Chapter 15: breeding and fishing pages: 257-268. MINEPIA/DEPC.
2. Mahieu M, Arquet R, Fleury J, Coppry O, Marie-Magdeleine C, Boval M, Archimede H, Alexandre G et Mandonnet N. 2009. Control of gastrointestinal parasitism ruminants grazing in humid tropical zones. *Ruminants* 16: 265-268.
3. Blake N et Coles G. 2007. Flock cull due to anthelmintic-resistant nematodes. *Veterinary Record* 161: 36.
4. Brunet S. 2008. Analysis of the antiparasitic action mechanisms of plants rich in polyphenolic substances on nematodes of the ruminant digestive tract. Thesis in view of obtaining a PhD from the University of Toulouse; 246p.
5. Chermette R. 1982. L'haemonchose ovine et ses particularités : importance and current situation in France. *Point Vétérinaire* 13: 21-28.
6. Dedehou VFGN, Olounladé PA, Adenilé AD, Azando EVB, Alowanou GG, Daga FD. 2014. *In vitro* effects of *Pterocarpus erinaceus* leaves and *Parkia biglobosa* fruit pods on two stages of the developmental cycle of *Haemonchus contortus* gastrointestinal nematode parasite of small ruminants. *J. Anim. Plant Sci.* 22(1): 3368-3378.
7. Qintin A, Frank J. 2004.Véterimary antihelmintics: old and new. *Trends Parasitol.* 20(10):456-61.
8. Fauvin, A. 2011. Molecular mechanisms involved in levamisole resistance in gastrointestinal strongyles. SCD de l'université de Tours, Tours, France.
9. Fajimi AK, Taiwo AA. 2005. Herbal remedies in animal parasitic diseases in Nigeria: a review. *African J. Biotechnol*. 4: 303-307.
10. WHO. 2013. Strengthening the role of traditional medicine in health systems: a strategy for the African region. WHO.

11. Uddin MK, Juraimi AS, Hossain MS, Nahar MA, Ali ME, & Rahman MM. 2014. Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. *The ScientificWorldJournal* 2014:951019.
12. Noreen S, Hussain I, Tariq MI, Ijaz B, Iqbal S, Qamar Ul Z, Ashfaq UA, Husnain T. 2015. *Portulaca oleracea* L. as a prospective candidate inhibitor of hepatitis c virus ns3 serine protease. *Viral Immunol*. 28:282–289.
13. Zoraghi R, Worrall L, See RH, Strangman W, Popplewell WL, Gong H, Samaai T, Swayze RD, Kaur S, Vuckovic M. 2011. Methicillin-resistant *Staphylococcus aureus* (MRSA) pyruvate kinase as a target for bis-indole alkaloids with antibacterial activities. *J Biol Chem*. 286(52): 44716–44725.
14. Chan K, MW Islam, M Kamil, R Radhakrishnan, MNM Zakaria, M Habibullah, A Attus. 2000. Analgesic and anti-inflammatory effect of *Portulaca oleracea*. *J. Ethnopharmacol*. 73: 445- 451.
15. Mensah JK, Ihenyen J and Iyamu M. 2013. Phytochemical and antimicrobial properties of *Emilia coccinea* (Cass). *Asian J. Contemp. Sci.* 2(2): 26-31.
16. Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achuki D, Nukenine E. N, Hensel A and Liebeu E. 2011. *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. *J. Helminthol*. 85: 304-312.
17. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ, World. 1992. Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.). Methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* 44:35-44.
18. Olounladé PA, Hounzangbé-Adoté MS, Azando EVB, Tamha TB, Brunet S,Moulis C, Fbre N, Fouraste I, Houste H. and Valentin A. 2011. *In vitro* study of the effect of *Newbouldia laewis* and *Zanthoxylum zanthoxyloides* tannins on the migration of infesting *Haemonchus contortus* larvae. *Int. J. Biol. Chem. Sci*. 5(4): 1414-1422.
19. Borsboom GJ, Boatin BA, Nagelkerke NJ, Agoua H, Akpoboua KL, Alley EW, Bissan Y, Renz A, Yameogo L, Remme J and Habbema JD. 2003. Impact of ivermectin on onchocerciasis transmission: assessing the empirical evidence that repeated ivermectin mass treatments may lead to elimination/eradication in West Africa. *Filaria J*. 2: 8-19.
20. Bidie AP, N'Guessan BB, Yapo AF, N'Guessan JD and Djaman AJ. 2011. Antioxidant activities of ten medicinal plants of the Ivorian pharmacopoeia. *Sciences et Nature* 8(1): 3-4.
21. Fankam AG, Kuete V, Voukeng IK, Kuiate JR and Pages JM. 2011. Antibacterial activities of selected Cameroonian spices and their synergistic effect with antibiotics against multidrug-resistant phenotypes. *BMC Complement Altern Med*. 11:104.
22. Karumi Y, Onyeyili PA, Ogugbuaja VO. 2004. Identification of active principles of *M. balsamina* (Balsam Apple) leaf extract. *J Med Sci*. 4(3): 179-182.
23. Santhi K, Sengottuvel R. 2016. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. *Int J Curr Microbiaol Appl Sci*. 5(1): 633-640.
24. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, Pinkas M, Luycky M, et Gazin M. 1996. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparation. *Arzneimittelforschung* 46(11):1086-9.
25. Boizot, N. Charpentier JP. 2006. *[Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier]. Cahier des Techniques de l'INRA*, pp.79-82.
26. Ba K, Tine E, Destain J, Cisse N and Thonart P. 2010. Comparative study of phenolic compounds, antioxidant power of different Senegalese sorghum varieties and amylolytic enzymes of their malt. *Biotechnol. Agro. Soc. Environ*. 14: 131-139.
27. Ayoola GA, Coker HAB, Adesegun SA, ADepeoju-Bello K Obaweya, Ezennia EC, Atangbayila. 2008. To. Phytochemical Screening and Antioxidant Activities of some selected Meedicinal plants used For Malaria Therapy in southwestern Nigeria. *Trop. J. Pharm. Res.*7(3): 1019-1024.
28. OECD. 2008. Repeated-dose 28-day oral toxicity study in rodents. In: OECD Guidelines for Testing of Chemicals. *Paris: OECD*. pp. 1-14.
29. Etamé LG, Yinyang J, Okalla EC, Makondo BV, Ngaba GP, Mpondo Mpondo E, Dibong SD. 2017. Acute and subacute toxicity study of wine extract of *Carica papaya* Linn seeds. *J Appl. Biosci*.120: 12077-12085.
30. Rhiouani, HJ EL-Hilaly, Israili ZH, Lyoussi B. 2008. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *J Ethnopharmacol.* 118: 378 – 386.
31. Lakmichi H, FZ. Bakhtaoui, CA Gadhi, A Ezoubeiri, Y EL-Jahiri, A EL-MansourI, I Zrara et K. Loutfi. 2011. Toxicity Profile of the Aqueous Ethanol Root Extract of *Corrigiola telephiifolia* Pourr. (Caryophyllaceae) in Rodents. *Evid Based Complement Alternat Med.* 2011: 317090..
32. Manda P, Manda O, Vangah-Manda MO, Kroa E, Djédjé Dano S. 2017. Study of acute and subacute toxicities of the nature remedy used in the treatment of malaria. *Rev. Ivoir. Sci. Technol*, 29 145 – 158.
33. Bancroft, J.D. and Gamble, M. eds., 2008. *Theory and practice of histological techniques*. Elsevier health sciences.
34. Hodge HC. et Sterner JH. 1943. Determination of substances acute toxicity by LDB50B. *Amer. Industrial Hyg. Assoc*. 10: 93.
35. Yongwa, G., Ngnoda, B.F.N.F., Ndjonka, D. and Saotoing, P., 2020. *In vitro* anthelmintic activity of aqueous and ethanolic extract of *Senna italica* (Caesalpiniaceae) on three-stages of *Haemonchus contortus*. *Journal of Pharmaceutical Research International* 32(3): 25-34
36. Assis, LM, Bevilaqua, CML. Morais, S.M. Vieira, LS. Costa, CTC.; Souza, JAL. (2003). Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmintica* Linn. extracts on *Haemonchus contortus*. *Vet. Parasitol*. 117: 43-4.
37. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol*. 99: 205-219.
38. Dobson RJ, Donal AD, Waller PJ, Snowdon KL. 1986. An egg hatch assay for resistance to levamisole in trichostrongyloid nematode parasites. *Vet Parasitol*.19(1-2):77-84.
39. Brunet S, Aufrere J, El Babili F, Fouraste I, Hoste H. 2007. The kinetics of ensheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both *in vitro* and *in vivo*. *Parasitology* 134(Pt 9):1253-62.
40. Bahuaud D, Martinez-Ortiz De Montellano *C*, *Chauveau* S, Prevot F, Torres-Acosta F, Fouraste I, Hoste H. 2006. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology* 132: 545-554.
41. Akkari H, Rtibi K, B'chir F, Rekik M, Darghouth, MA & Gharbi M. 2014. *In vitro* evidence that the pastoral *Artemisia campestris* species exerts an anthelmintic effect on *Haemonchus contortus* from sheep. *Veterinary Research Communications* 38: 249–255.
42. Hoste H, Jackson F, Athanasiadou S, Thamsborg SM & Hoskin, SO. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* 6: 253–261.
43. Marie-Magdeleine C, Udino L, Philibert L, Bocage B, Archimedes H. 2010. *In vitro* effect of cassava (*Manihot esculenta*) leaf extracts on four developmental stages of *Haemonchus contortus*. *Vet. Parasitol*. 173: 85-92.
44. brahim, AM. 1992. Anthelmintic activity of some Sudanese anthelmintic plants. *Phytother Res.* 6: 155-157.
45. Fiorucci S. 2006. Biological activities of flavonoid family compounds: approaches by quantum chemical and molecular dynamics methods. PhD thesis. University of Nice; 211p.
46. Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen Kaur. 2011. Phytochemical screening and Extraction. A Review. *Internationale Pharmaceutica Sciencia*. 1: 1Ŕ9.
47. OECD. 2001. Subcommittee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals. Health and Environmental Hazards - Acute Toxicity. UN/SCEGHS/2/INF.11, 13p.
48. Betti HA HA betti, AC Stein, E Dallegrave, AT Barth Wouters, TT Negrão Watanabe, D Driemeier, A Buffon, et MS Kuze Rates. 2012. Acute and repeated-doses (28 days) toxicity study of *Hypericum polyanthemum* Klotzsch ex Reichardt (Guttiferare) in mice. *Food Chem Toxicol*. 50: 2349–2355.
49. Raza, M Al-Shabanah OA, El-Hadiyah, TM, Al-Majed, AA. 2002. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss *albino* mice. *Scientia Pharmaceut.* 70 :135–145.
50. WHO. 2000. General methodological principles for research and evaluation relating to traditional medicine WOH/TRM/2000, 1 Annex II. pp: 31-35.
51. Peirs C. 2005. Contribution to the phytochemical study of *Galega officinalis* L. (Fabaceae). Doctoral thesis in Pharmacognosy, Institut National Polytechnique de Toulouse, *Toulouse, France*, 130p.
52. Marieb, EN. 2009. Essentials of Human Anatomy and Physiology (9e éd.), San Francisco (CA), Pearson/Benjamin Cummings, 632 p.
53. Wilson, DD, S Lahaye, J Courchesne and E. 2010. Prégent. Examens paracliniques, Montreal, *Chenelière/McGraw-Hill*, 696 p.
54. Clarke, WC, and Y Nagahama. 1977. Effect of premature transfer to sea water on growth and morphology of the pituitary, thyroid, pancreas, and interrenal in juvenile coho salmon (*Oncorhynchus kisurch*). *Can. J. Zool.* 55: 1620-1630.
55. Gueguen J, Godart N, Chambry J, Brun-Eberentz A, Foulon C, Divac PhD, SM Huas, C. 2012. Severe anorexia nervosa in men: Comparison with severe AN in women and analysis of mortality. *International Journal of Eating Disorders* 45(4): 537–545.
56. Sennlaub Florian, Yves Courtois, and Olivier Goureau. 2001. Inducible nitric oxide synthase mediates the change from retinal to vitreal neovascularization in ischemic retinopathy. *J Clin Invest*. 107(6):717-725.