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DOUKOURE Maya
Laboratoire de Physiologie Animale Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso

TINDANO Basile
Laboratoire de Physiologie Animale Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso

DA F. Léonard
Laboratoire de Sciences de la Vie et de la Terre, Unité de Formation et Recherches en Sciences et Technologies, Université Norbert Zongo, Koudougou, BP: 376, Burkina Faso

ZABRE Géneviève
École Normale Supérieure, Koudougou, Burkina Faso

BAYALA Balé
Laboratoire de Physiologie Animale Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso

BELEMTOUGRI G. Raymond
Laboratoire de Physiologie Animale Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso

Correspondence:
Dr. TINDANO Basile
Laboratoire de Physiologie Animale Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso
Email: tind3bas1@gmail.com

Antioxidant potential, toxicity, and effect of *Calotropis procera* extract on milk production in Wistar rats

DOUKOURE Maya, TINDANO Basile, DA F. Léonard, ZABRE Géneviève, BAYALA Balé, BELEMTOUGRI G. Raymond

ABSTRACT

Breast-feeding failure is a public health concern, as the use of breast milk substitutes increases the risk of morbidity and mortality among infants in developing countries. In Burkina Faso, people rely on medicinal plant to treat Breast-feeding failure. Indeed, *Calotropis procera* is medicinal plant used in traditional medicine to treat Breast-feeding failure. The phytochemical components, safety and antioxidant activity of the plant were first determined. Aim of this study was to evaluate the effects of aqueous extract of *C. procera* on milk supply. The effect of *C. procera* on milk supply was evaluated in lactating rats by measuring pup weight during suckling period. At the start of the experiment, lactating females weighing 241.22 ± 9.64 g were divided into three lots of five rats each. The lot I was administered with NaCl (0.9%), the lot II was treated with metoclopramide (5 mg/kg b.w.) and lot III received aqueous extract of *C. procera* at the dose of 200 mg/kg b.w. The drug was administered orally and started from the evening (18:00) of day 3 of lactation to day 17.

The phytochemical components such as steroids and triterpenoids, flavonoids, cardenolids, tannins, saponosids and reducing sugars were detected in the plant extracts. The aqueous and hydro-ethanolic extracts showed a weak antioxidant activity. In the acute test no signs of toxicity and mortality were recorded. In the Sub-acute test, any signs of toxicity were observed in rat during the period of treatment. There was no significant change in Hematological and biochemical parameters between the lots treated with extract and the control lot. The aqueous extract (200 mg/kg) of *C. procera* increased milk production significantly ($p < 0.01$) compared to blank control (NaCl, 0.9%). The milk production increased by 39%. Conclusion: Aqueous extract of *C. procera* can stimulate milk supply in rats and therefore confirm its use in traditional medicine in the treatment of mother's milk insufficiency.

Keywords: *Calotropis procera*, Innocuity, Lactogenic effect, Rat.

INTRODUCTION

In Africa, especially in Burkina Faso, most people rely on traditional medicine through medicinal plants to treat many usual diseases. Lactogenic plants are part of the medicinal plants used by women with lactation insufficiency [1,2]. Indeed, lactogenic plants are believed to induce, stimulate or maintain maternal milk production [3,4]. Some scientific works showed that these plants are able to stimulate the secretion of lactogenic hormones such as prolactin, growth hormone, cortisol. Moreover, lactogenic plants induce accumulation of β-casein in *vivo* and in *vitro* conditions [1,5].

C. procera is a species belonging to Asclepiadaceae family. The plant is drought-resistant, salt-tolerant, high about 6 m and all parts exude the latex when it is cut [2,6]. The whole plant or different parts were used in the Indian traditional medicine to treat leprosy, ulcers, tumor, piles and the diseases of spleen, liver and abdomen. The plant is also used as a purgative and anthelmintic, the latex is considered as abortifacient [7,8]. Moreover, the leaves, the bark, the root are active against some genital diseases such as blennorrhea or gonorrhea, menorrhagia, dystocia. These parts are also used in the treatment of agalactia and hypoagalactia in women and animal [2,9]. The aim of this study was to validate the traditional use of *C. procera* as lactogenic plant in Burkina Faso.

MATERIALS AND METHODS

Collection and authentication of plant

The branch leaves of *C. procera* were harvested in the morning (6 am to 11 am) in the province of Comoé especially in Banfora in 2014. The plant material was washed with copious amounts of water and dried under artificial ventilation away from sunlight and dust. The dried branch leaves of the plant were

reduced to powder using an electric grinder. The plant was identified in Herbarium of University Joseph KI-ZERBO under number 16968.

Preparation of extracts

For the preparation of aqueous extract, one hundred (100) gramme of *C. procera* powder were decocted in 700 mL of distilled water. After boiling during one hour (1h), the mixture was filtered successively by pressure on fine nylon cloth and on cotton wool. The filtrate was concentrated and then lyophilized.

Concerning the hydro-alcoholic extract, one hundred (100) were boiled in 700 mL of hydro-alcoholic solution (80% of ethanol). The decoction was filtered through whatman filter paper N°5. The decocted was concentrated under reduced pressure in the rotary evaporator at temperature of 50-60°C and dried within sweating-room during twenty-four (24) hours. The extracts were kept in the refrigerator for use.

Animal model

Female Wistar rats deriving from animal house of University Joseph KI-ZERBO were used for all experimentation. They were kept under standard conditions, with a temperature of 22 ± 3 °C, a photoperiod of 12 hours of light and 12 hours of darkness and a relative humidity of 50 ± 10%. The animals were fed protein-enriched pellets and had free access to water.

Phytochemical screening

The phytochemical components of aqueous and hydro-alcoholic extracts were identified according to [10]. Indeed, steroids and/or triterpenoids, tannins, flavonoids, saponosids, alkaloids, anthraquinones, coumarins, anthocyanosids, cardenolids, gum and mucilages, reducing sugars were researched through colorimetric tests.

TOXICITY STUDIES

Acute toxicity study

The acute toxicity study was carried out according to OCDE guideline [11]. Nine female Wistar rats weighing 150.09 ± 12.40 g and aged ten (10) weeks were used. The animals were divided into three groups of three rats each. The group I received distilled water and served as control group. The groups II and III were given a single dose of 2000 mg/kg body weight (b.w.) of aqueous and hydroethanolic extracts of *C. procera*, respectively and served as test groups. All animals were observed during for (04) hours, twenty-four (24), forty-eight (48) and seventy-two (72) hours After administration. The different toxicity signs and death were noted.

Sub-acute toxicity study

This study was achieved according to the method described in OCDE guidelines [12] with slight modifications. Twenty-four nulliparous female rats weighing 118.05 ± 6.90 g and aged eight weeks were used. After allotment, the animals were divided into four groups of six rats each. Group I received distilled water and served as the control group. Groups II, III and IV were taken aqueous extract of *C. procera* (EACP) at doses at doses of 50, 100 and 200 mg/kg body weight respectively. The extract was administered daily at the same time for 28 consecutive days. The behaviour of the animals was observed daily throughout the treatment period. All rats were weighed weekly during the experimental period. Twenty-four (24) hours after the last administration the animals were sacrificed and autopsied. They were previously anaesthetized with a mixture of ketamine/xylazine (1/0.7) in order to collect blood by cardiac puncture.

For the haematological analysis, the blood was collected into EDTA (Ethylene Diamine Tetraacetate) tubes and the blood cells count were achieved using an automatic counter of haematology. Moreover, blood samples were collected into the dry tubes and centrifuged at 3500 rpm for 5 min and the serum obtained were used for biochemical analysis. The methodology of Spinreact were used for spectrophotometric determination of the different biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, cholesterol and total proteins. After blood collection, the organs such as liver, lung, spleen, heart, kidney, uterus, ovary and adrenal gland were taken and weighed.

ANTIOXIDANT ACTIVITY:

2,2 diphenyl-1-picrylhydrazyl (DPPH) radical assay

The principle of this test is based on the extract's ability to reduce the DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical, which is violet in colour and turns yellow after reduction. This discolouration is measured using a spectrophotometer at 517 nm. The antioxidant activity of aqueous and hydroethanolic extracts of *C. procera* was assessed according to the method of [13] with a few modifications.

To achieve this, ascending concentrations of the aqueous (5 - 400 µg/mL) and hydroethanolic (0.5 - 40 µg/mL) extracts of the plant were prepared. Then, one hundred microlitres (100 µL) of the aqueous and hydroethanolic extracts of the plant were added to 200 µL of DPPH (2 mg/mL). A positive control was prepared by adding 100 µL of quercetin or ascorbic acid (0.05 - 4 µg/mL) to 200 µL of DPPH. A negative control was made up of 200 µL DPPH and 100 µL methanol. Each mixture was homogenized and incubated in the dark at room temperature for 15 minutes. Absorbances were read using a spectrophotometer at 517 nm. The percentage of DPPH radical scavenging was calculated from the formula : DPPH radical scavenging activity (%) = $[(AC_{517} - AE_{517})/AC_{517}]*100$

AC_{517} is the absorbance of a DPPH solution without extract

AE_{517} is the absorbance of the tested plant extract with DPPH

The test was carried out in triplicate and the mean IC50 values (concentration resulting in 50% inhibition) were determined graphically.

Ferric reducing power (FRAP) assay

The FRAP method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by reducing components. The ability of extract to reduce ferric ion to ferrous ion was assessed using the method described by [14].

For this purpose, test tubes containing 0.5 mL of extract with a concentration between 0.1 and 1 mg/mL were added to 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium hexacyanoferrate $[K_3Fe(CN)_6]$ were also added. The whole was mixed and heated to 50°C in boiling water for 30 minutes. Then, 1.25 mL of trichloroacetic acid (10%) was added to the mixture and the whole was centrifuged at 2000 rpm for 10 minutes. A volume of 125

µL of the supernatant was removed and mixed with 125 µL of distilled water and 25 µL of freshly prepared 0.1% $FeCl_3$ in 96 microwells plate. Absorbances were read at 700 nm against a standard curve of acid ascorbic ($y = 0.0749 x + 0.1569$; $r^2 = 0.992$) which was pre-established using a series of concentrations (0.003 to 0.1 mg/mL). Quercetin and gallic acid were used as positive controls. All tests were performed in triplicate.

Lipid peroxidation inhibition

The lipid peroxidation inhibition activity of the extract was determined using the 2-thiobarbituric acid method described by [15] and [16]. Iron sulphate heptahydrate $FeSO_4$, $7H_2O$ and hydrogen peroxide H_2O_2 were used to induce lipid peroxidation of liver homogenate and spleen brain.

To perform this assay, 0.2 mL of aqueous and hydroethanolic extracts of *C. procera* (1.5 mg/mL) were mixed with 1.0 mL of liver or brain homogenate in 1% Tris-HCl buffer (50 mM, pH 7.40). Then 50 µL $FeSO_4$ (0.5 mM) and 50 µL H_2O_2 (0.5 mM) were added. The mixture was incubated at 37ºC for 60 minutes, before adding 1.0 mL trichloroacetic acid (15%) and 1.0 mL 2-thiobarbituric acid (0.67%). The resulting mixture was heated to 100ºC in boiling water for 15 minutes and the absorbances were read at 532 nm using a spectrophotometer. Ascorbic acid was used as the reference component.

Effect of aqueous extract of *C. procera* on milk production

Milk production in lactating rats was assessed using the method described by [5]. Fifteen (15) adult primiparous rats were used. They were weighed and mated (1 male/2 females). Two weeks later, the pregnant rats were removed and placed individually in cages until parturition. Twenty-four (24) hours after parturition, the pups from each parturient rat were adjusted to nine (9) pups per lactating animal. Nursing rats with an average weight of 214.22 ± 9.64 g were divided into three groups of 5 animals each. Group 1 was treated with NaCl (0.9%) and served as a blank control, group 2 was treated with Metoclopramide (5 mg/kg) and constituted the positive control. Group 3 received 200 mg/kg body weight of aqueous extract of *C. procera.* Treatment was carried out orally from day 3 to day 17 of lactation from 18 :00 GMT. Milk production was measured from day 4 to day 17 of lactation. The yield of milk production and the weight of the offspring were measured each day. For the estimation of milk production 18 hours and 23 hours after treatment, the pups were weighed five (05) times each day during the experimental period.

At 7 :00 am the pups were weighed (W1) and immediately isolated from the lactating females for 4 hours. At 11:00, they were weighed again (W2) and returned to the lactating rats to suckle for one hour. At 12:00, they were removed from the lactating females to be weighed (W3) before being isolated again for 4 hours. At 16:00 the pups were weighed (W4) and returned to the lactating rats to nurse for one hour. At 17 :00 they were finally weighed (P5) and returned to the female rats for the night. Milk production 18 hours after administration of the extract was calculated according to the formula W3-W2 with a correction for weight loss due to metabolic processes (W2-W1)/4. Milk production 23 hours after gavage was also calculated using the following formula : W5-W4 with a correction [(W2-W1) + (W4-W3)]/8.

Statistical analysis

Data were presented as mean ± standard error of mean (SEM) and analyzed by using Graph Pad Prism version 5.03. One-way analysis of variance (ANOVA) followed by Dunnett's comparison test were used to assess differences between groups. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening

The phytochemical components present in *C. procera* extracts were showed in table 1. Different constituents including tannins, steroids and triterpenoids, flavonoids, cardenolids were detected.

Table 1: Phytochemical screening of the branch leaves extracts of *C. procera*

Phytochemical components	Aqueous extract	Hydroethanolic extract
Tannins	+/-	+
Steroids and triterpenoids	++	++
Anthraquinones	-	+/-
Coumarins	-	-
Alkaloids	-	-
Flavonoids	+	++
Saponosids	+/-	+
Cardenolids	+	++
Anthocyanosids	-	-
Gum and mucilage	-	-
Reducing sugars	-	+

Toxicity studies

Acute toxicity of aqueous and hydroethanolic extracts of *C. procera*

The aqueous and hydroethanolic extracts of the plant did not cause any mortality in rats 72 h after a single oral administration. In addition, no behavioral changes were observed in animals which received both extract 14 days after treatment.

Sub-acute toxicity of aqueous extract of *C. procera*

The doses 50, 100 and 200 mg/kg of the aqueous extract of *C. procera* did not induce mortality during all the period of treatment. No behavioral changes were noted in all animals treated with extract compared to control group.

Body and organs weight

An increase of body weight in all animals was observed during the time of experimentation. All animals body weight increased. However no significant difference was observed between the body weight gain of the rats treated with extract compared to control (Figure 1).

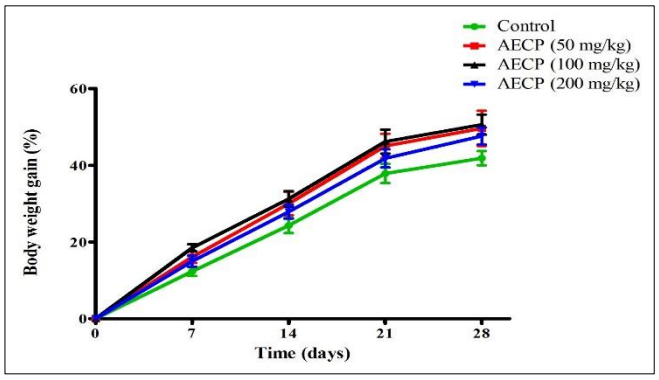

Figure 1: Effect of aqueous extract of *C. procera* on body weight gain in rats.

No significant change was observed between the relative weight of liver and kidney of animals exposed to extract compared to control (Figure 2).

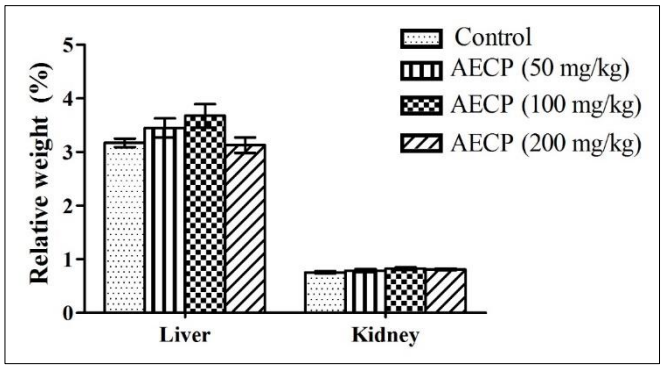

Figure 2: Effect of aqueous extract of C. procera on relative weight of liver and kidney. No significant ($p > 0.05$) difference was noted between the relative weight of the organs in control group compared to extract groups.

Concerning the relative weight of lung, there was a dose dependent increase. For this organ a significant increase ($p < 0.05$) was noted at the dose of 200 mg/kg. However, no difference ($p > 0.05$) was observed in treated and control rats for their heart and spleen relative weight (Figure3).

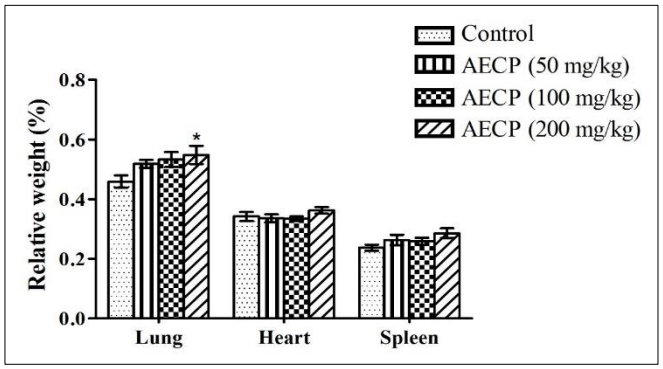

Figure 3: Effect of aqueous extract of *C. procera* on relative weight of lung, heart and spleen.

The relative weight of uterus, ovary and adrenal glands was presented in figure 4. There was a slight decrease of relative weight of uterus at the doses 100 and 200 mg/kg. Concerning, the relative weight of ovary and adrenal glands, no significant ($p > 0.05$) change was recorded.

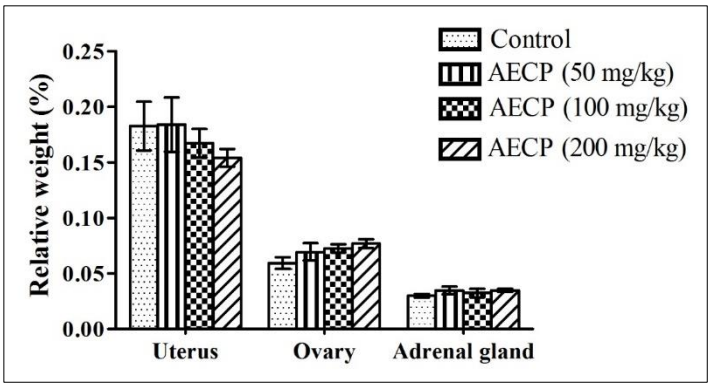

Figure 4: Effect of aqueous extract of *C. procera* on relative weight of uterus, ovary and adrenal glands. There was no significant change of relative weight in animals.

The results of hematological parameters of the treatments on extract and control related were presented in table 2. The extract did not affect the hematological parameters.

Table 2: Effect of aqueous extract of *C. procera* on hematological parameters of the animals in sub-acute toxicity

Parameters	Doses (mg/kg/day b.w.)			
	Control	**50**	**100**	**200**
WBC (10^3/µL)	1.75 ± 0.40	1.83 ± 0.28	2.35± 0.73	2.16 ± 0.60
GRAN (%)	17.83 ± 2.12	21.5 ± 2.33	18.75 ±3.09	19.2 ± 1.77
LYM (%)	75.50 ± 2.18	72.00 ± 2. 43	74.50 ± 2.90	74.40 ± 1.66
RBC (10^6/µL)	7.21 ± 0.12	7.57 ± 0.12	7.58 ± 0.15	7.16 ± 0.09
HCT (%)	40.87 ± 0.88	40.98 ± 0.63	39.84 ± 0.24	38.80 ± 0.52
HGB g/Dl	15.06 ± 0.31	15.37 ± 0.24	15.20 ± 0.15	14.63 ± 0.16
PLT (10^3/µL)	537.9 ± 54.81	607.5 ± 20.17	571.6 ± 21.60	523.5 ± 18.59
MPV (fL)	5.95 ± 0.08	6.01 ± 0.08	5.96 ± 0.15	5.98 ± 0.11

The table 3 shows the results of biochemical parameters. No significant change was noted between treated and control groups.

Table 3. Effect of aqueous extract of *C. procera* on biochemical parameters of animals in sub-acute toxicity

Parameters	Treatment			
	Doses (mg/kg/day b.w.)			
	Control	50	100	200
ALT (UI/L)	42.32 ± 3.53	44.48 ± 2.84	47.77 ± 4.64	39.43 ± 2.59
AST (UI/L)	143.9 ± 19.34	132 ± 18.27	164.2 ± 16.64	142.3 ± 20.43
Creatinine (µmol/L)	63.68 ± 3.55	66 ± 3.39	52.2 ± 4.88	65.45 ± 6.06
Urea (mmol/L)	7.97 ± 0.31	9.23 ± 0.51	9.04 ± 0.47	7.04 ± 0.25
Total Protein (mmol/L)	5.52 ± 0.13	5.65 ± 0.12	5.58 ± 0.11	5.45 ± 0.12
Cholesterol (mmol/L)	1.29 ± 0.11	1.28 ± 0.08	1.01 ± 0.12	1.19 ± 0.12

The result of antioxidant activity of the hydroethanolic extract of *C. procera* were presented in table 4. Taking account all the three methods, the extract exhibited a weak power antioxidant compared to reference molecules. The IC_{50} value of extract was 102.66 ± 11.23 or

even ninety-fold lower than quercetin. Nevertheless, the plant showed a potent capacity to inhibit a lipid peroxidation in liver (57.43 ±1.28%) compared to standard ascorbic acid. Aqueous extract showed any antioxidant activity.

Table 4: Antioxidant activity of hydroethanolic extract of *C. procera*

	DPPH (IC_{50} (µg/mL))	FRAP (mmol EAA/100g)	Lipid peroxidation inhibition (%)	
			Brain	Liver
Ascorbic acid	0.62 ± 0.13	-	98.02 ± 0.01	98.02 ± 0.01
Quercetin	1.13 ± 0.05	505.27 ± 15.77	-	-
Gallic acid	-	601.06 ± 24.49	-	-
hydroethanolic extract	102.66 ± 11.23	7.20 ± 0.69	22.55 ± 0.62	57.43 ± 1.28

Effect of aqueous extract of *C. procera* on milk production in rats

Milk production of treated and controls (blank and reference) rats is shown in figure 5. A significant increase ($p < 0.01$) of milk production was observed in rats exposed to extract at 200mg/kg and that 23 h after administration. Milk yield increased from 0.45 ± 0.04 to 1.11 ± 0.11 g/pup/day for the blank control. Concerning rats treated with metoclopramide (positive or reference group), milk production increased from 0.51 ± 0.05 to 1.20 ± 0.08 g/pup/day. The extract at 200 mg/kg increased milk production from 0.73 ± 0.10 to 1.54 ± 0.11 g/pup/day in rats 23 h after administration.

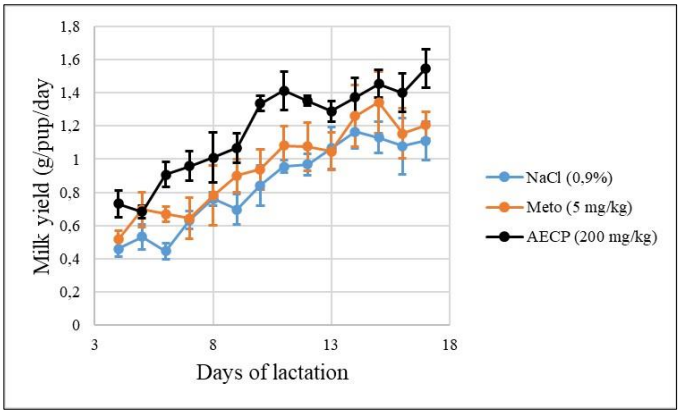

Figure 5: Effect of aqueous extract *C. procera* on milk production 23 h after administration in Wistar rats.

The mean of milk production of treated and control animals were presented in figure 6. It was 0.84 ± 0.06; 0.95 ± 0.06 and 1.18 ± 0.07 g/pup/day for the blank, metoclopramide and extract groups, respectively. Mean milk production was higher ($p < 0.01$) in extract treated group compared to the blank control.

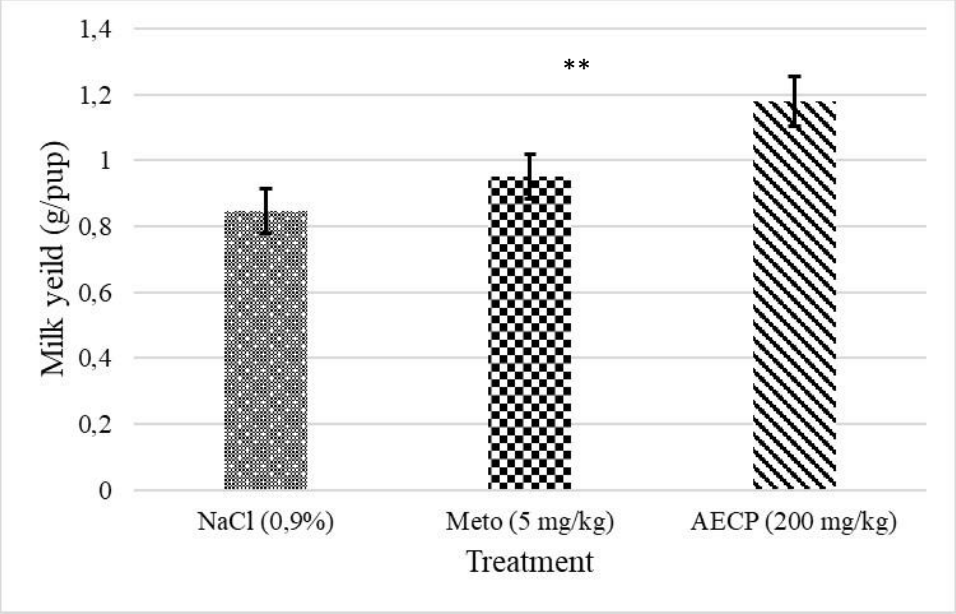

Figure 6: effect of aqueous extract of *C. procera* on mean milk production per day.

An increase of body weight was observed in all pups during the period of experimentation. Indeed, the body weight of pup increased from 8.38 ± 0.37 to 28.47 ±1.21 g/pup for the blank control. Concerning the metoclopramide treated group, the pup body weight increased from 7.87 ± 0.16 to 29.97 ± 1.37 g/pup. In the extract treated group, pup weight increased from10.44 ± 0.70 to 33.34 ± 1.80 g/pup (figure 7). No significant change ($p > 0.05$) in body weight of pup of mothers was observed.

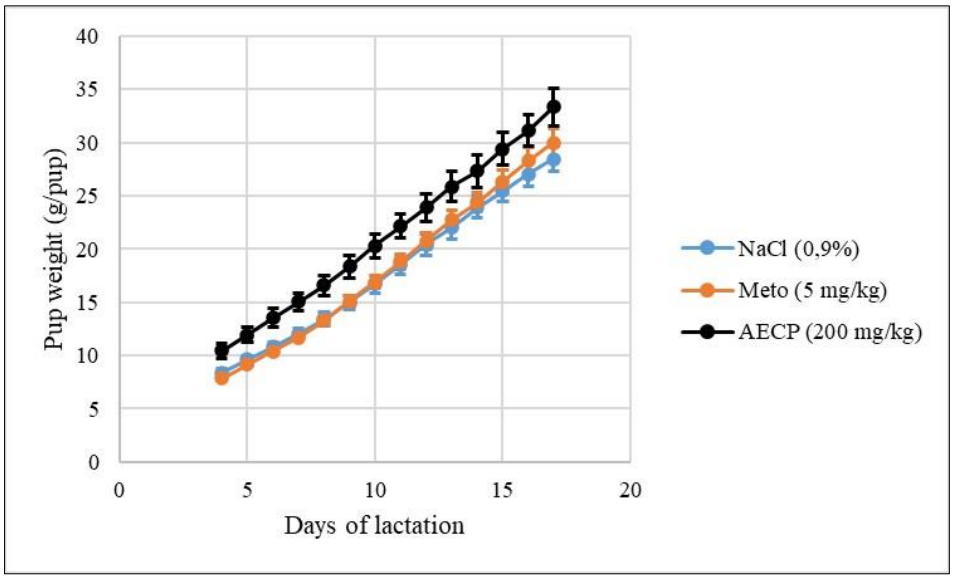

Figure 7: Effect of aqueous extract of *C. procera* on pup body weight 23 h after treatment.

DISCUSSION

The branch leaves of *C. procera* contain some compounds such as steroids and triterpenoids, flavonoids, cardenolids, tannins, saponosids and reducing sugars. The results are in agreement with the finding of [17,18]. Moreover, the presence of these components may be explained the medicinal use of this plant in mother's milk insufficiency [19,20].

Acute toxicity of aqueous extract of *C. procera* were evaluated on Wistar adult's rats. The results showed that extract did not cause any death and no signs of toxicity at 2000 mg/kg b.w. These results suggested that the LD_{50} of this plant was higher than 2000 mg/kg b.w. Our results are similar to those of [21] who showed that the aerial parts and roots barks extracts of *C. procera* do not cause toxic effects or mortality at the dose of 3000 and 2000 mg/kg b.w., respectively.

Subacute toxicity study of aqueous extract of *C. procera* at the doses of 50, 100 and 200 mg/kg b.w. did not cause signs of toxicity and mortality in rats. Indeed, body weight increase or body weight gain were observed for all animals during the period of experimentation. This body weight gain was due to the food and water intake and suggested that the extract did not induce the loss of appetite or did not adversely interfere with the nutritional benefits [22]. Our results are in agreement with the findings of other authors who also observed the body weight gain in rabbits and rats treated with aqueous extracts of the leaves and roots barks of *C. procera*, respectively [23,21]. In addition, the relative weight of the organs (liver, kidney, heart, spleen, uterus, ovary and adrenal glands) of extract treated groups was similar to control group. Similar results were found by [21] with aqueous extract of roots barks of *C. procera.* Nevertheless, a significant increase of relative weight of lungs at the dose of 200 mg/kg b.w. was observed. This result suggests that the extract could affect lung structure. According to [24], lung weight increase could be associated with lung damage in subacute inhalation study. Further histological study could furnish more information regarding the lung toxicity of the extract.

The haematological and biochemical parameters are analyzed to evaluate the toxic effects of the extract on haematopoietic system and some organs functions. In the present study, no significant changes in haematological parameters were noted between treated and control groups. These results suggest that the extract did not affect the production of blood cells. Our results are in agreement with those of [21] who have found that the roots barks extract of *C. procera* administrated to rats during six weeks does not cause significant effect on haematological parameters.

Liver and kidney are two important organs which play a key role in metabolic processes in maintaining body homeostasis. According to [25,26] hepatorenal function tests are very important for toxicity evaluation of the drugs and plants extracts. Thereby, no significant difference was recorded in biochemical parameters between treated and control animals. The insignificant changes of levels of serum transaminases (ALT, AST), creatinine and urea are good indicators of liver and kidney function [27,28]. Therefore, the no significant change in ALT, AST and total protein suggests that the extract did not induce liver damage in rats. Furthermore, the creatinine and urea level are the most widely used and accepted as good method of evaluation of renal function [29,30]. In our study, the creatinine and urea level of treated groups was similar to control group. These results showed that the aqueous extract of *C. procera* could not have deleterious effects on kidney. [31] found the same results on serum biochemical parameters with the aqueous suspension of dry latex of *C. procera.*

Several methods have been used for the antioxidant activity evaluation. On the whole, hydroethanolic extract of branch leaves of *C. procera* showed average antioxidant activity. Our results are similar to those of [32,33] who found that methanol extract of leaves, aerial part and latex of *C. procera* possess antioxidant activity. This antioxidant activity of hydroethanolic extract of *C. procera* may be due to the presence of some phytochemical compounds as tannins and flavonoids [34,35]. Oxidative stress is responsible of several diseases for both mothers and infants. Indeed, some health disorders such as cancer, cardiovascular diseases, diabetes, neurodegenerative diseases and mastitis are associated to oxidative stress [36,37]. For infants, oxidative stress is involved in lung and intestinal diseases [38,39]. Some researches showed the presence of some phytochemical compounds in mother's milk which can prevent or reduce oxidative stress in both mothers and infant [40,41]. Similar compounds as flavonoids were present in our extracts of *C. procera* and may be some potent antioxidant agents. Therefore, they could contribute to fight oxidative stress in both mothers and infants.

Breastfeeding is the natural and main way to feed newborn infants. Health benefits of breastfeeding for both infants and mothers are well known. Mother's milk insufficiency is the most common reason of discontinuation of breastfeeding. For this reason, women with milk insufficiency use medicinal plants to induce or increase milk production.

Milk production was significantly higher in aqueous extract treated group than untreated group. According to [42], milk production is correlate with the number of mammary epithelial cells and their secretory activity in the gland. Therefore, in our study the significant increase of milk production in lactating rats treated with aqueous extract of *C. procera* may be explained by the effect of extract on mammary gland. In other words, the extract could stimulate the mammary epithelial cells proliferation and their activity. Furthermore, the increase of number or activity of mammary secretory cells are regulated by the endocrine system and the physiological state [42]. Some researchers found that some lactogenic plants stimulate the secretion of some hormones involving in lactation such as prolactin, growth hormone and cortisol or induce β-casein accumulation in the mammary gland [43,5]. In addition, [44] showed that PRL and GH are the major regulators of milk production. Indeed, PRL maintains milk synthesis by inhibiting epithelial cells loss, maintaining cellular differentiation and have effect on biochemical processes involved in the synthesis of the major compounds of milk whereas GH acts directly on mammary gland to stimulate milk synthesis. On the other hand, our extract could have an effect on the lactogenic hormones synthesis which stimulate milk production.

There is a linear increase of body weight of suckling pups during the period of experimentation. However, no significant change was observed in body weight gain of all pup. Our results are in agreement with to those of [45]. According to these authors, milk production is not necessarily correlated with body weight gain.

CONCLUSION

The aqueous and hydro-ethanolic extracts of *C. procera* contain steroids and triterpenoids, flavonoids, cardenolids, tannins, saponosids and reducing sugars. The acute and subacute toxicity studies in rats showed that the aqueous extract of *C. procera* is practically non-toxic at the dose use and safe orally. Moreover, the hydro-ethanolic extract of *C. procera* showed a weak antioxidant activity. The plant increase milk production in rats which confirms its use in traditional medicine for the treatment of mother's milk insufficiency.

Conflict of interest

The authors declare no conflicts of interest

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