Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/7524
DOI URL: http://dx.doi.org/10.21474/IJAR01/7524

RESEARCH ARTICLE

PHYTOCHEMICAL COMPOSITION, TOXICITY, ANTIOXIDANT AND LACTOGENIC ACTIVITIES OF *EUPHORBIA HIRTA* (L.).

[1]Doukouré Maya, [1]Bayala Balé, [2]Guenné Samson, [1]Tindano Basile and [1]Belemtougri Raymond.

1. Laboratoire de Physiologie Animale (LA.PA), Université Ouaga I Pr. Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03 (Burkina Faso).
2. Laboratoire de Biochimie et de Chimie Appliquées (LA.BIO.C.A), Université Ouaga I Pr. Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03 (Burkina Faso).

Manuscript Info

Manuscript History

Received: 10 June 2018
Final Accepted: 12 July 2018
Published: August 2018

Keywords:-
Lactogenic activity, Euphorbia hirta, Toxicity, Antioxidant, Milk production**.**

Abstract

Breastfeeding is widely recognized to have many health benefits for both infants and mothers and in developing countries, women rely on medicinal plants to increase breast milk production. *Euphorbia hirta* is one of the medicinal plants used in Burkina Faso for the treatment of lactation insufficiency. The purpose of this study was to investigate its effect on milk production. Previous studies were achieved to identify phytochemical compounds, to assess innocuity and antioxidant activity of the plant. Oral acute and sub-acute toxicity were carried out as described by Organization for Economic Cooperation and Development (OECD) 2001 and 2008, respectively. Several methods including Diphenylpicrylhydrazyl (DPPH), Ferric reducing power (FRAP), Lipid peroxidation were used to evaluate antioxidant activity. The effect of *Euphorbia hirta* on milk production was assess in lactating rats. Preliminary phytochemical screening revealed the presence of steroids and triterpenoids, tannins, flavonoids, coumarins, anthocyanosids, reducing sugars. The plant exhibited strong antioxidant potential. Limit test of 2000 mg/kg b.w. did not cause any mortality and no signs of acute toxicity suggesting that the plant is practically non-toxic. Concerning sub-acute toxicity, no mortality and no signs of toxicity were observed in all the extract treated groups similarly to control group. Haematological and biochemical parameters were not affected by the extract, therefore the plant is safe for use when it is administered orally. The extract treated rats produced higher milk than blank and reference controls. The body weight gain of the pups was 2.40±0.08, 2.81±0.22 and 2.78±0.28 for blank, reference controls and extract treated group, respectively. *Euphorbia hirta* contains several phytochemical compounds which may stimulate milk production in mammalian.

Introduction:-

Breastfeeding is widely recognized to have many health benefits for infants and mothers. Indeed, for infants breastfeeding is well-known to have protective effects against infectious diseases and elevated systolic blood

Corresponding Author:- Doukouré Maya.
Address:- Laboratoire de Physiologie Animale (LA.PA), Université Ouaga I Pr. Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03 (Burkina Faso).

pressure. In addition, it was associated with higher performance on intelligence tests and cognitive development. Breastfeeding also reduces the risk of overweight, obesity, type I and II diabetes in childhood and later in adults. Moreover, for nursing mothers, breastfeeding give protection against premenopausal breast cancer, ovarian cancer, obesity and type II diabetes. It improves the birth spacing, reduces the risk of hyperlipidemia and cardiovascular diseases (Danforth *et al*., 2007; Binns *et al*., 2016; Victora *et al*., 2016). In view of the health benefits for both infants and nursing mothers, WHO and UNICEF recommend exclusive breastfeeding during the first six (6) months of life and continued breastfeeding until 24 months of age (WHO, 2009). Despite the benefits of breast milk, only 37% of infants younger than 6 months are exclusively breastfed in low-income countries (Victora *et al*., 2016). The most common reason of this low rate of breastfeeding throughout the world is the mother's milk insufficiency (Sjolin *et al*., 1977; Gatti, 2008; Sultana *et al*., 2013). In developing countries, breast milk substitutes is generally associated with increase of morbidity and mortality's risk in infants and it's the most common cause of malnutrition. Therefore, lactation insufficiencies constitute a public health concern (Sultana *et al.*, 2013). Women with lactation failure or lactation insufficiency use the pharmaceutical galactogogues or lactogogues which are substances that induce, maintain and increase breast milk production. Unfortunately, the majority of these drugs cause some side effects in mother and in infants (Sultana *et al.*, 2013; Zuppa *et al*., 2010). Hence, the use of natural products like the medicinal plants could be an alternative to alleviate the lactation disturbances especially lactation insufficiency.

Euphorbia hirta L. (*E. hirta*) is one of the medicinal plants used in the traditional medicine to increase milk production in women in Burkina Faso (Sawadogo, 1987; Doukouré *et al*., 2018).

It is a small annual herb reaching 40 cm high and belonging to the family Euphorbiaceae. The plant is native to Australia but it is spreaded in all the tropical and subtropical countries. *E. hirta* is very solicited in the traditional medicine for treatment of several illness such as digestive system diseases, respiratory system diseases, and genital apparatus diseases like agalactia, liver and heart diseases. This plant is also used for various ocular ailments and affections of skin (Lanhers *et al*., 1990; Lanhers *et al*., 2005). In view of large utilization of the plant in traditional medicine, the present study was carried out to evaluate its innocuity, its antioxidant activity and its effect on milk production.

Materials and Methods:-

Collection of plant material

Whole plants of *E. hirta* were collected in the morning at 6 to 11 AM in May 2014 in Banfora, province of Comoé in the Cascades region. The plant sample was washed with tap water, dried under ventilation at room temperature. The dried sample of the plant were pulverized using a mechanical grinder. The powder obtained was used for extraction.

Preparation of extracts

One hundred (100) gramme of *E. hirta* whole plant powder were boiled during one hour (1h) in 700 mL of distilled water and 700 mL of hydro alcoholic solution (80% of ethanol) for aqueous and hydro-ethanolic extracts preparation, respectively. After cooling, the mixture of aqueous extract was filtered through tissue wool and absorbent cotton. The filtrate obtained was concentrated in a sweating room, lyophilized and packaged in bottle.

The hydro-alcoholic extract was filtered through whatman filter paper N°5. The filtrate obtained was concentrated by rotary evaporator under reduced pressure at temperature between 50-60°C and dried within sweating-room during twenty-four (24) hours. The aqueous and hydroethanolic extracts were stored in the refrigerator (+4° C) until use.

Animal model

Adult Wistar female rats were obtained from the animal house of University Ouaga I Pr Joseph KI-ZERBO. The animals are bred in the standard conditions including temperature 22 ±3 °C with photoperiod 12h light/12h dark cycle and humidity at 50 ± 10%. The animals are fed with industrial pellets with 29% protein and have water ad libitum. All the tests in this work were performed according to the protocols already approved by the Department of Animal Physiology of University Ouaga I Pr Joseph KI-ZERBO and met the international standards of animal study (Zimmermann, 1983).

Phytochemical screening
The phytochemical screening of aqueous and hydroethanolic extracts of *E. hirta* was carried out according to Ciulei (1982). The phytochemical compounds such as steroids and/or triterpenoids, tannins, flavonoids, saponosids, alkaloids, anthraquinones, coumarins, anthocyanosids, cardenolids, gum and mucilages, reducing sugars were checked.

Toxicity Studies:-
Acute toxicity study
The acute toxicity test was conducted according to OECD guideline (OECD, 2001). Adult nulliparous and no pregnant Wistar rats weighing between 146 and 184 g, aged ten (10) weeks were used. A single oral dose of 2000 mg/kg body weight (b.w.) of aqueous extract of *E. hirta* (AEEH) was administered to three rats. After treatment, the animals were observed individually during one (01) hour and daily fourteen (14) consecutive days. The mortality and the signs of toxicity such as the changes of skin, fur, and eyes were recorded.

Sub-acute toxicity study
The sub-acute toxicity test was carried out according to OECD guidelines (OECD, 2008) with some modifications. Twenty-four nulliparous and no pregnant Wistar rats weighing between 106 and 131 g, aged eight (08) weeks were used. The rats were randomized into four groups of six animals each. The group I served as control and received distilled water. The groups II, III, IV were treated with the aqueous extract of *E. hirta* (AEEH) at the doses of 50, 100, 200 mg/kg b.w., respectively. The extract was administered orally to all animals daily during twenty-eight (28) days. During the period of treatment all animals were weighed weekly. Toxicity signs, mortality and body weight changes were recorded. At the end of the treatment, the rats were fasted overnight. Twenty four (24) hours after, the animals were anesthetized with a mixture of ketamine and xylazine (1/0.7) by intraperitoneal injection and blood samples were obtained by cardiac puncture for haematological and biochemical analysis.

For haematological analysis, the blood was collected into EDTA (Ethylene Diamine Tetraacetate) tubes. The haematological parameters including red blood cells (RBC), white blood cells (WBC), haematocrit (HCT), haemoglobin (HGB), lymphocytes (LYMPH), granulocytes (Gran), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean platelet volume (MPV) were determined using an automatic counter of haematology (Mindray BC 3000PLUS).

For biochemical analysis, blood samples were collected into the dry vacutainer tubes and centrifuged at 3500 rpm for 5 min. The sera obtained were used for the test. 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, internal organs such as liver, lung, spleen, heart, kidney, uterus, ovary and adrenal gland were removed and weighed.

Antioxidant Activity:-
Diphenylpicrylhydrazyl (DPPH) radical assay
Diphenylpicrylhydrazyl (DPPH) is a free radical with violet color. When it is mixed with a substance that can donate a hydrogen atom or an electron, it was reduced to a pale yellow color due to the acceptance of hydrogen or an electron.

The stable free radical 2, 2 diphenyl-1-picrylhydrazyl was used for *in vitro* determination of free radical scavenging activity of aqueous and hydroethanolic extracts of *E. hirta*. In a 96 microwells plate, 100µL of different concentrations (0.05-40µg/mL) of each extract was added to 200µL of DPPH solution (2mg/mL). The mixture was incubated in dark and at room temperature to complete the reaction for 15 min. Quercetin and ascorbic acid were used as reference compounds. The free radicals scavenging activity of extracts was evaluated using spectrophotometer at 517 nm. The pourcentage of discoloration was calculated by following 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

IC_{50} was determined for 50 % inhibition concentration using a graph. All tests were performed in triplicate.

Ferric reducing power (FRAP) assay
The reducing power of extracts was determined according to Hinneburg *et al*. (2006).

This reduction concerns extract ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). For this evaluation, 0.5 mL of extract of different concentrations (0.1-1 mg/mL) were mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% aqueous potassium hexacyanoferrate [K_3F_e $(CN)_6$]. The mixture was incubated at 50°C for 30 min. In the mixture, 1.25 mL of trichloroacetic acid (10%) was added to stop the reaction and the total mixture was centrifuged at 3000 rpm for 10 min. After, 125 µL of upper layer was mixed with 125 µL of distilled water and 25 µL of F_eCl_3 (0.1%) fresh solution in 96 microwells plate. Ascorbic acid was used to produce a calibration curve by reading absorbences at 700 nm ($y=0.0749x + 0.1569$, $r^2=0.992$). Quercetin and gallic acid were used as positive controls. All tests were performed in triplicate.

Lipid peroxidation inhibition

Lipid peroxidation assay was carried out using the method of Ohkawa *et al*. (1979) adapted by Guenné *et al*. (2012). The lipid peroxidation was induced by $FeSO_4$, $7H_2O$ and H_2O_2 in the normal rat liver and brain homogenate. Zero points two (0.2) millilitres of aqueous and hydroethanolic extracts of *E. hirta* (1,5 mg/mL) were mixed with 1 mL of rat liver or brain homogenate in 1 % of Tris-HCl buffer (50 mM; pH 7, 4) then, 50 µL $FeSO_4$ (0.5 mM) and 50 µL of H_2O_2 (0.5 mM) were added. The mixture was incubated at 37 °C for 60 min. After incubation, 1 mL of trichloroacetic acid (15%) and 1 mL of thiobarbituric acid (0.67%) were added to the mixture and heated in boiling water for 15 min. The lipid peroxidation inhibition was measured at 532 nm using a spectrophotometer. Ascorbic acid was used as standard.

Effect of aqueous extract of *E. hirta* on milk production

The effect of *E. hirta* on milk production of rats was evaluated as described by Lompo-Ouédraogo *et al*. (2004). Fifteen (15) nulliparous female rats were used. The animals were mated with male in plastic cages in the standard conditions of breeding until pregnancy. Two (02) weeks after mating, all the pregnant rats were housed individually in plastic cages. Following birth, the litters were adjusted to nine pups per dam. At the beginning of lactation, the animals weighed 241.22±9.64 g and were divided into three experimental groups of five lactating dams each. The group I received NaCl (0.9%) and served as blank control, the group II treated with metoclopramide (5mg/kg b.w.) served as reference control and group III was given aqueous extract of *E. hirta* at the dose of 200 mg/kg b.w. The administration was done orally by gavage. All the animals were treated daily, starting from the evening (17 h 00) of day 3 of lactation to day 17. Milk production was measured from day 4 to day 17 of lactation. Milk yield, weight of pups, body weight gain of pups were determined each day. Milk production was estimated 18 h and 23 h after treatment. Each day during the period of experimentation, the pups were weighed at 7 h 00 (w1) and isolated from their mother for 4 h. At 11 h 00, the pups were weighed (w2) and returned to their mother and allowed to feed for 1 h. At 12 h 00 they were weighed again (w3). Milk yield 18 h after treatment was estimated as w3-w2 with a correction for weight loss due to metabolic processes (urination, defection and respiration) in the pup as (w2-w1)/4. For the estimation of milk yield 23 h after the treatment, the pups were subsequently isolated at 12 h 00 for 4 h. At 16 h 00 they were weighed (w4) and reunited with their mother for 1 h of feeding. Finally the pups were weighed (w5) again at 17 h 00 and left with their mother to the night. Milk yield 23 h after treatment was calculated as w5-w4 with a correction for weight loss due to metabolic processes in the pups as [(w2-w1) + (w4-w3)]/8.

Statistical analysis

Data are expressed as mean ± standard error of mean (Mean ±S.E.M.). The data obtained were analyzed using Graph Pad Prism version 5.03. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test were used to determine differences between the groups. P-value <0.05 was considered as statistically significant.

Results:-

Phytochemical screening

The qualitative phytochemical screening of aqueous and hydroethanolic extracts of whole plant of *E. hirta* revealed the presence of compounds which are recorded in the table I.

Table I:- Phytochemical screening of aqueous and alcoholic extracts of *E. hirta*

Phytochemical compounds	Aqueous extract	Hydroethanolic extract
Steroids and triterpenoids	+	+
Tannins	++	++
Flavonoids	+/-	++
Saponosids	+/-	+/-

Alkaloids	-	-
Anthraquinones	-	-
Coumarins	+	++
Anthocyanosids	+/-	+
Cardenolids	-	-
Gum and mucilages	+/-	+/-
Reducing sugars	++	++

++ = abundant, + = average, +/- = trace, - = absent

Toxicity Studies:-

Acute toxicity of aqueous extract of *E. hirta*

No mortality was observed after oral administration of single dose of 2000 mg/kg b.w. of extract during 72h. No signs of toxicity were observed. The result of acute toxicity indicated that the LD_{50} is more than 2000 mg/kg.

Sub-acute toxicity

Oral administration of aqueous extract of *E. hirta* at the doses of 50, 100 and 200 mg/kg b.w. did not cause any mortality or morbidity in rat during 28 days of treatment. In addition, no signs of toxicity were observed in all the extract treated groups compared to control group.

During the period of treatment, the increase of body weight was noted in animals treated with the extract similarly to control group ($p > 0.05$) (Fig 1).

The relative organ weight of liver, kidney, lung, heart, spleen, uterus, ovary and adrenal gland are presented in Fig 2, 3 and 4. These results show that there was no significant difference ($p > 0.05$) in organ weight of extract treated groups compared to control group. Nevertheless, a slight dose-dependent decrease of relative weight of uterus was observed.

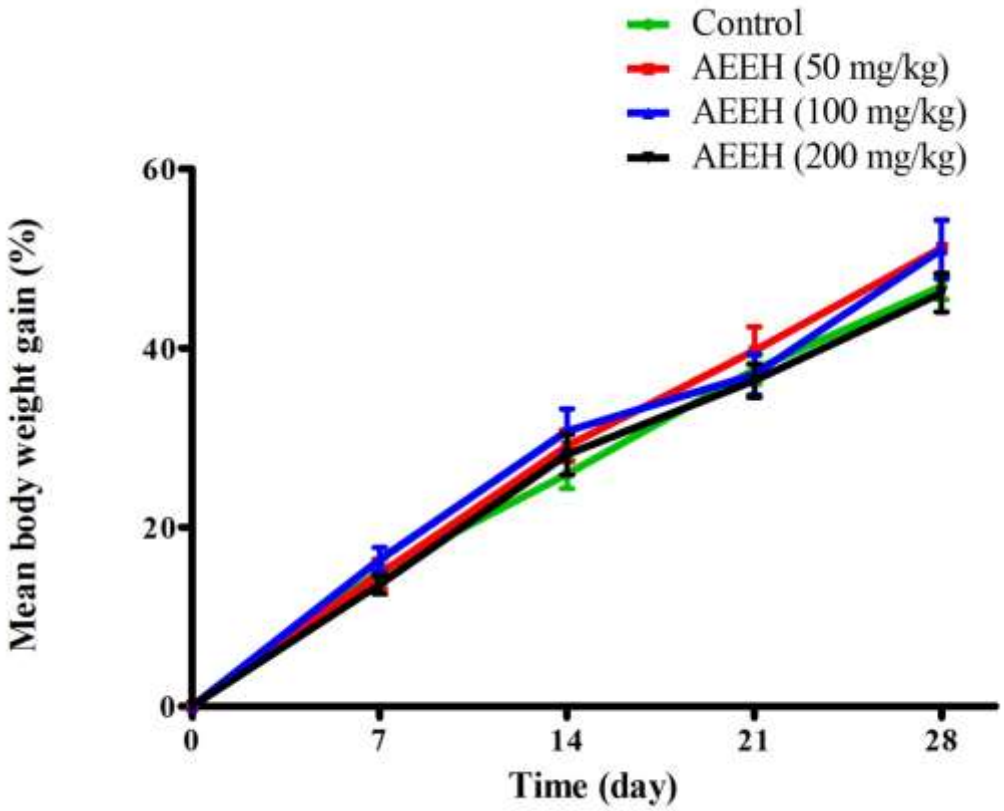

Fig 1:- Body weight gain of control group and AEEH treated groups. There was an increase of body weight in all extract treated groups similarly to control group.

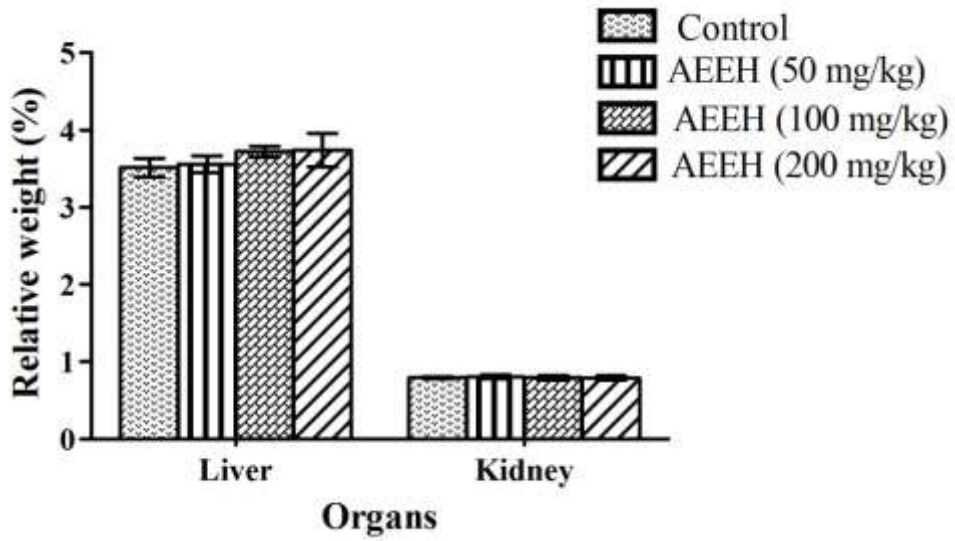

Fig 2:-Relative weight of liver and kidney of control group and AEEH treated groups. There was no significant difference ($p > 0.05$) in organ weight of extract treated groups compared to control group.

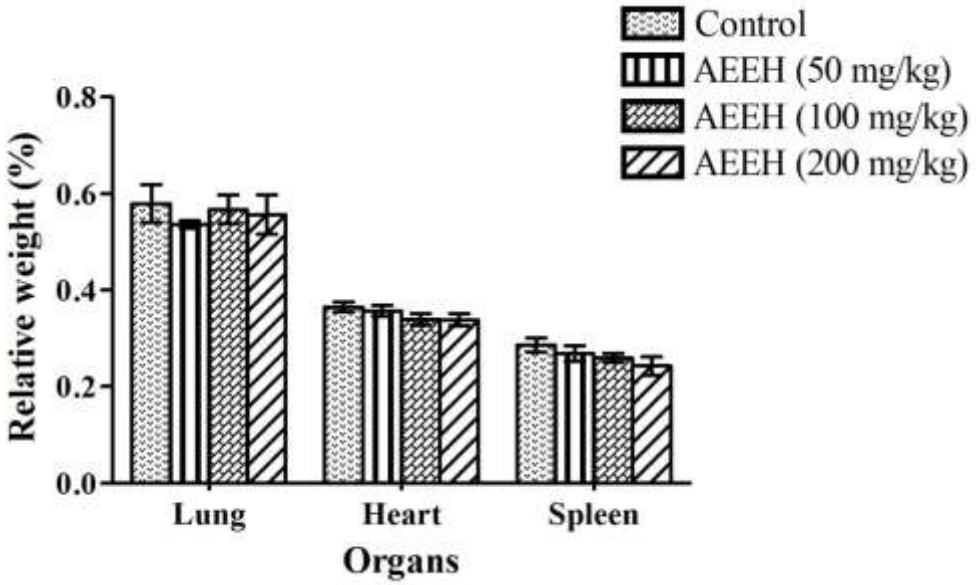

Fig 3:- Relative weight of lung, heart and spleen of control group and AEEH treated groups. There was no significant difference ($p > 0.05$) in organ weight of extract treated groups compared to control group

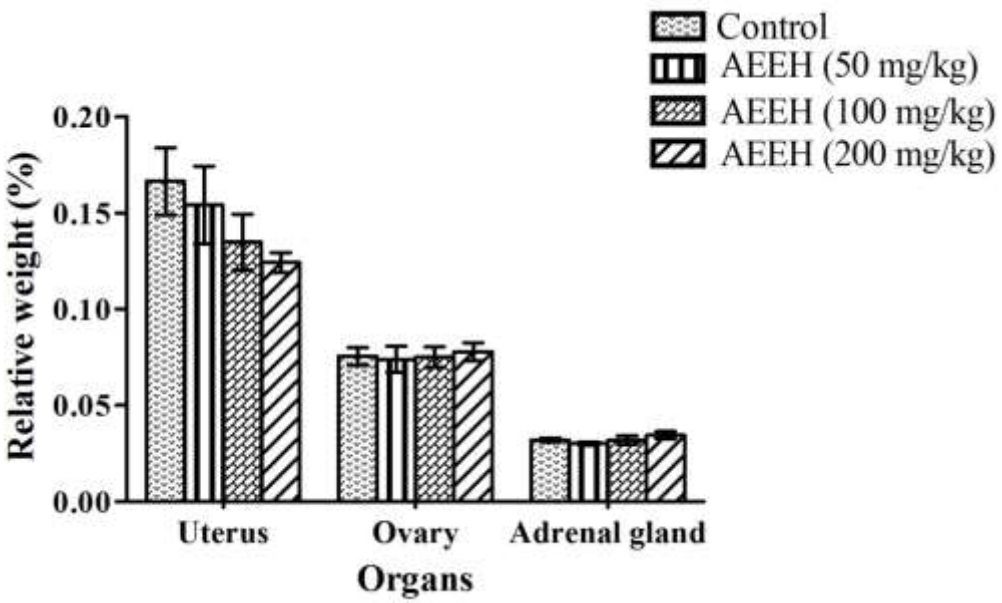

Fig 4:- Relative weight of uterus, ovary and adrenal gland of control group and AEEH treated groups. There was no significant difference ($p > 0.05$) in organ weight of extract treated groups compared to control group

Effect of AEEH on haematological and biochemical parameters

There were no significant changes ($p > 0.05$) between control and the treated groups at the doses of 50 and 100 mg/kg. A significant decrease ($p < 0.05$) of red blood cells level at the dose of 200 mg/kg was noted compared to control (Table II).

Table II: -Effect of AEEH on haematological parameters of Wistar rats in sub-acute toxicity

Parameters		Doses (mg/kg/day b.w.)		
	control	50	100	200
WBC (10^3/µL)	2.30 ± 0.51	2.88 ± 0.53	1.64 ± 0.20	2.35 ± 0.34
Gran. (%)	19.83 ± 2.21	18.00 ± 2.40	19.40 ± 2.24	18.50 ± 2.78
Lymph. (%)	73.83 ± 2.14	75.00 ± 2.81	74.00 ± 2.58	75.00 ± 3.18
RBC (10^6/µL)	7.39 ± 0.03	7.31 ± 0.13	7.50 ± 0.13	6.95 ± 0.12 *
HCT (%)	40.55 ± 0.41	40.12 ± 0.77	40.82 ± 0.34	38.18 ± 0.91
MCV (fL)	54.85 ± 0.45	54.93 ± 0.17	54.52 ± 0.58	54.93 ± 0.48
MCH (pg)	20.30 ± 0.10	20.38 ± 0.13	20.62 ± 0.27	20.60 ± 0.16
HGB (g/dL)	15.05 ± 0.10	14.93 ± 0.33	15.5 ± 0.14	14.38 ± 0.35
PLT (10^3/µL)	581.70 ± 22.29	630.00 ± 19.35	576.60 ± 23.01	567.00 ± 35.12
MPV (fL)	5.90 ± 0.05	5.85 ± 0.06	6.06 ± 0.08	5.97 ± 0.14
MCHC (g/dL)	37.10 ± 0.23	37.15 ± 0.24	37.92 ± 0.14	37.60 ± 0.25

The results of biochemical analysis showed that at the doses of 100 and 200mg/kg b.w., no significant difference were observed compared to control. A significant decrease ($p < 0.01$) of creatinine level was observed at the dose of 50mg/kg b.w. compared to control (table III).

Table III:- Effect of AEEH on biochemical parameters of Wistar rats in sub-acute toxicity.

Parameters	Treatments			
		Doses (mg/kg/day b.w.)		
	Control	50	100	200
ALAT (UI/L)	49.86 ± 1.66	49.43 ± 4.71	42.36 ± 3.82	59.92 ± 8.57
ASAT (UI/L)	123.50 ± 19.27	127.30 ± 19.79	154.40 ± 22.14	199.90 ± 25.35
Urea (mmol/L)	9.15 ± 0.40	8.43 ± 0.18	8.61 ± 0,56	8.86 ± 0.34

Creatinine (µmol/L)	63.33 ± 3.07	52.22 ± 1.96**	55.64 ± 2.37	55.64 ± 1.35
Total Protein (mmol/L)	5.99 ± 0.09	5.83 ± 0.09	5.91 ± 0.08	6.01 ± 0.08
Cholesterol (mmol/L)	0.93 ± 0.17	1.08 ± 0.14	0.95 ± 0.05	0.96 ± 0.18

Antioxidants Activity:-

DPPH radical assay

The hydroethanolic extract of *E. hirta* exhibited a potent DPPH radical scavenging activity compared to aqueous extract. The IC_{50} value of hydroethanolic extract was 4.93 ± 0.40 µg/mL and that of aqueous extract was 46.33 ± 3.21 µg/mL. Overall, the extracts of *E. hirta* presented a lower antioxidant activity compared to ascorbic acid and quercetin which have 0.62 ± 0.13 and 1.13 ± 0.05 as $IC_{50,}$ respectively (table IV). Indeed, scavenging activity of free radicals of hydroethanolic extract of *E. hirta* is seven-fold lower than that of ascorbic acid whereas the aqueous was seventy four-fold less potent than ascorbic acid.

Table IV:- IC_{50} values of different extracts of *E. hirta*

Group	IC_{50} (µg/mL)
Aqueous extract	46.33 ± 3.21
Hydroethanolic extract	4.93 ± 0.40
Quercetin	1.13 ± 0.05
Ascorbic acid	0.62 ± 0.13

Ferric reducing power determination (FRAP)

The hydroethanolic extract exhibited the high reducing activity than the aqueous extract. Both extracts showed less reducing activity than the quercetin and gallic acid (tableV).

Table V: -Determination of reducing power of *E. hirta*

Group	Ferric reducing property (mmol AAE/100g)
Aqueous extract	38.96 ± 0.45
Hydroethanolic extract	54.49 ± 7.96
Quercetin	505.27 ± 15.77
Gallic acid	601.06 ± 24.49

Lipid peroxidation inhibition in rat brain and liver

All extracts had the property to reduce lipid peroxidation. Nevertheless, the extracts showed a more potent capacity to decrease lipid peroxidation in liver homogenate than in brain (table VI).

Table VI:-Lipid peroxidation inhibition of the extracts in rat brain and liver

	Lipid peroxidation inhibition (%)	
Group	Brain	Liver
Control	100	100
Ascorbic acid	98.02 ± 0.01	98.02 ± 0.01
Aqueous extract	38.72 ± 0.34	78.61 ± 0.50
Hydroethanolic extract	42.11 ± 0.28	80.08 ± 0.34

Effect of aqueous extract of *E. hirta* on milk production in rats

The effect of *E. hirta* on milk production are presented in Fig 5. The extract induced a non-significant increase ($p > 0.05$) of milk production in rats comparatively to control groups (NaCl and metoclopramide).

Milk yield increased from 0.45 ± 0.04; 0.51 ± 0.05 and 0.65 ± 0.08 g/pup/day to 1.11 ± 0.11; 1.20 ± 0.08 and 1.40 ± 0.11 g/pup/day, respectively for the blank, reference controls and *E. hirta* extract group.

The mean milk production was 0.84 ± 0.06; 0.95 ± 0.06 and 1.04 ± 0.07 g/pup/day respectively for the blank, reference controls and *E. hirta* extract group (Fig 6). The extract did not cause an increase of milk production 18 h after the treatment compared to the control. Whereas, 23h after the treatment a non-significant ($p > 0.05$) increase of milk production was recorded.

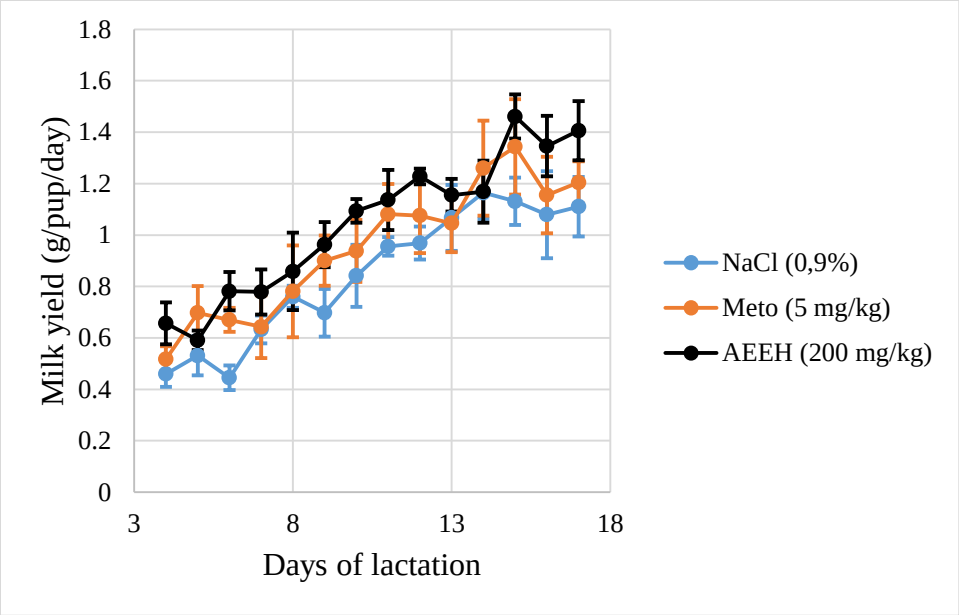

Fig 5:- Effect of AEEH on milk production 23h after the treatment. Values are expressed as mean ± SEM. There was a non-significant ($p > 0.05$) increase of milk production in extract treated rats comparatively to controls groups. (Meto: metoclopramide, Eh: *Euphorbia hirta*)

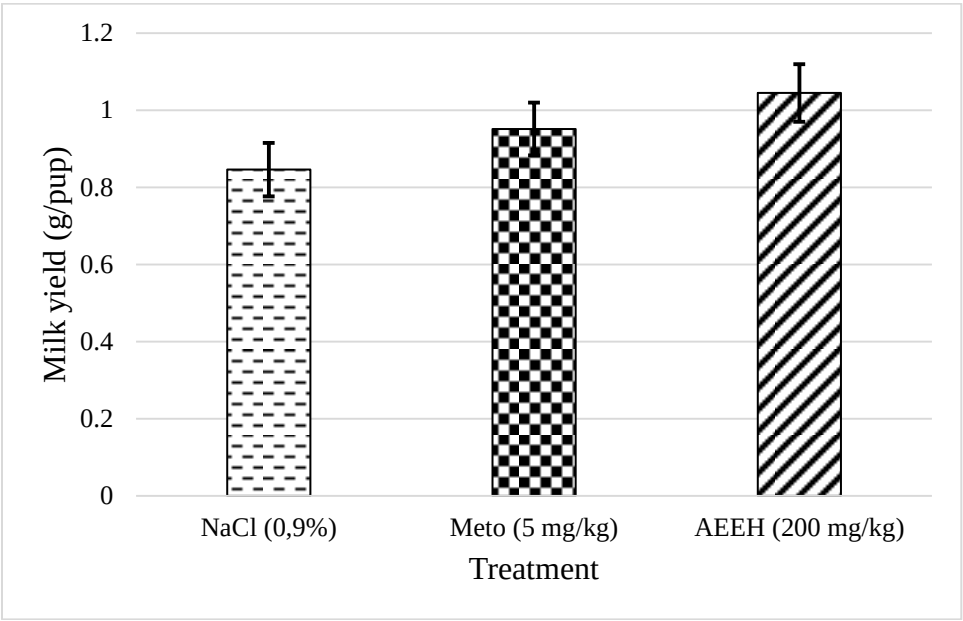

Fig 6:- Mean milk production per day. There was a non-significant ($p > 0.05$) increase of milk yield between the extract treated group and the controls.

Body weight of pups

The body weight increase from 8.38 ± 0.37; 7.87 ± 0.16 and 7.68 ± 0.64 g/pup/day to 28.47 ±1.21; 29.97 ± 1.37 and 28.42 ± 1.39 g/pup/day respectively for the blank, reference controls groups and extract treated group (figure 7). The body weight gain of the pups was 2.40 ± 0.08; 2.81 ± 0.22 and 2.78 ± 0.28 for the blank, reference controls and extract treated group, respectively (figure 8). There were no significant ($p > 0.05$) changes in body weight of pups between all groups but a slight body weight gain was observed for reference and extract treated groups compared to blank control.

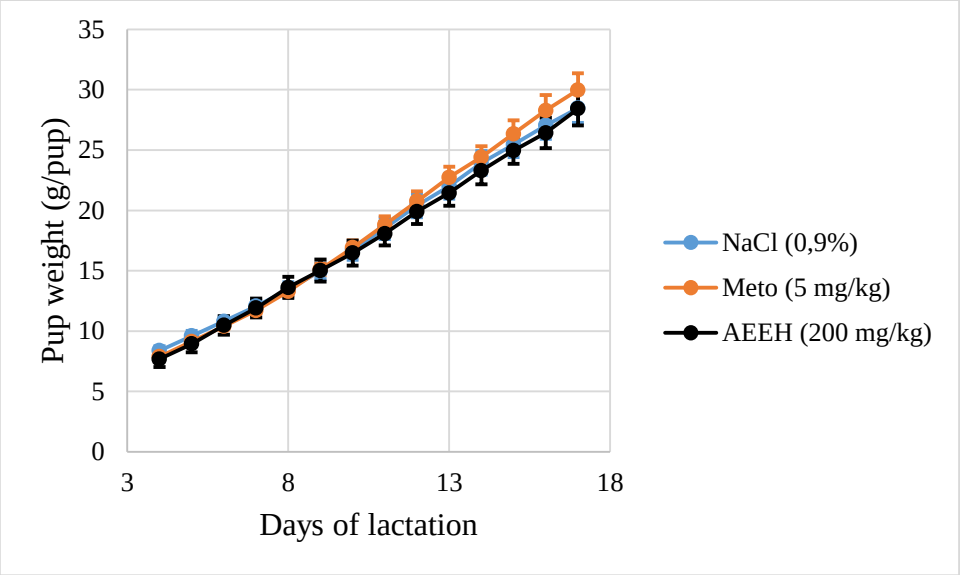

Fig 7:- Effect of AEEH on pups' weight 23h after administration. There were no significant ($p > 0.05$) changes in body weight of pups between the different groups

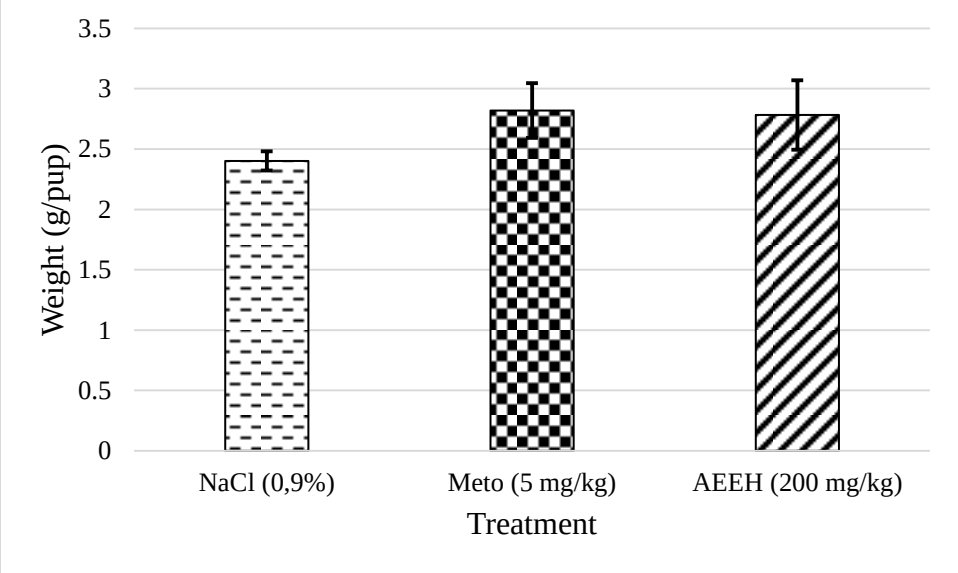

Fig 8: -Mean weight gain of pups. A non-significant body weight gain was observed between the different groups

Discussion:-

Plants use as alternative to increase milk production in women is an approach currently practiced by women in Cascades region of Burkina Faso. The main objective of this study was to evaluate the effect of *E. hirta* on female rat milk production, to confirm the traditional use of this plant.

Phytochemical screening

The preliminary phytochemical screening revealed the presence of phytochemical compounds such as tannins, steroids and triterpenoids, coumarins, flavonoids, anthocyanosids, reducing sugars. These results corroborate those of Shih and Cherng (2006) and Qaisar *et al*. (2012). Moreover, Deleke koko *et al*. (2011) showed that some lactogenic plants contain tannins, terpenoids and steroids, flavonoids, saponosids, anthocyans, alkaloids cardenolids, quinone. Therefore, the presence of these phytochemical compounds in the plant could justify its medicinal use in the lactation.

Toxicity

The acute and sub-acute toxicities of aqueous extract of *E. hirta* were evaluated on Wistar rats in order to detect the harmful effects on human health. Indeed, the single dose of 2000 mg/kg b.w. did not cause any death and no signs of toxicity.

The result of acute toxicity showed that the LD_{50} was higher than 2000 mg/kg b.w. According to the scale of Hodge and sterner (1943), the extract is classified as a slight toxic drugs. Our results are similar to those of Ping *et al*. (2013) who showed that the extract of *E. hirta* does not cause an acute toxicity effect at the dose of 5000 mg/kg b.w. and then the DL_{50} is more than 5000 mg/kg b.w.

The effect of the extract after a long-term treatment (28 days) was evaluated. The oral administration of aqueous extract of *E. hirta* at the doses of 50, 100, 200 mg/kg b.w. did not show any morbidity and mortality in rats. In addition no signs of toxicity were recorded. Moreover, the increase of body weight or body weight gain were observed for all animals (treated and control groups). Our results are in agreement with the findings of other authors who also observed the body weight gain in rats treated with *E. hirta* (Ping *et al*. 2013). According to these authors this weight gain is due to the food and water intake suggesting that the extract did not induce the loss of appetite during the period of the experiment. Futhermore, the extract did not adversely interfere with the nutritional benefits maintaining the appetite and led to the body weight gain. Subsequently, there was no significant changes in the relative weight of the internal organs of extract treated animals compared to control except the uterus where slight dose dependent decrease of relative weight was observed. These results are similar to those of Ping *et al*. (2013) who found no significant changes in relative weight of internal organs.

The haematological and biochemical analysis were carried out to determine the effect of the extract on the internal organs functions. For haematological parameters, no significant changes were noted at the dose of 50 and 100 mg/kg b.w. while at the dose of 200 mg/kg b.w. a significant decrease of red blood cells was observed. This decrease of red blood cells is an indicator of anemia which may be due to bone marrow dysfunction or suppression (Mohajeri *et al*., 2007). This result suggests that the extract at this dose and beyond may cause anemia. According to Kluwe (1981) and Olorunnisola *et al*. (2012) the functionality of liver and kidney is very important for toxicity evaluation of the drugs and plants extracts. Thereby, most of the biochemical parameters were not affected by the extract. The insignificant changes of the levels of transaminases (ALT, AST), creatinine and urea are good indicators of liver and kidney function (Jaouad *et al.*, 2004; Diallo *et al*., 2010). Therefore, the no significant change in ALT, AST and total protein suggests that the extract did not induce liver damage in rats. The findings of other researchers who showed that the ethanolic extract of *E. hirta* has the hepatoprotective property support our result (Tiwari *et al*., 2011; Dubey and Mehta, 2012). Furthermore, the creatinine and urea level is the most widely used and accepted as good method of evaluation of renal function (Wasan *et al*., 2001; Ouédraogo *et al*., 2013). Thus, an increase of creatinine level is an indicator of renal injury (Vyas and Argal, 2012). The extract caused a decrease of creatinine level at the low dose of 50mg/kg b.w. which could be considered as a non-toxic effect of the plant. Further, the histological examinations could confirm the non-toxic effect on the internal organs.

Antioxidant activity

The results obtained suggest that the extracts of *E. hirta* had strong antioxidant potential. These results are similar to those of kandalkar *et al*. (2010); Basma *et al*. (2011); Bakr *et al*. (2012) who found that *E. hirta* possesses antioxidant activity. Some health disorders like mastitis which can affect all lactating mammals, occurs during the early of lactation generally the first month, seems to be related to oxidative stress. The mastitis is an inflammation of mammary gland which is associated with release of free radicals causing a decrease of antioxidant and an increase of oxidant in milk (Weiss, 2005; Yang and Li, 2015). The antioxidants which protect the body from free radicals either by scavenging them or by inhibiting the activity of oxidizing enzymes, play an important role in the prevention or the treatment of mastitis (Yang and Li, 2015). In addition the antioxidants supplementation in cattle have positive effect on milk quantity and quality (Castillo *et al*., 2013; Kahkeshani *et al.*, 2015). The antioxidant activity of *E. hirta* may be explained by the presence of phytochemical compounds such as flavonoids, tannins (Okamura *et al*., 1993; Amarowicz *et al*, 2000; Majewska *et al*., 2011)

Effect of aqueous extract of *E. hirta* on milk production in rat

Milk production is a complex physiological process involving physical and emotional factors and the interaction of several hormones especially prolactin (PRL) and growth hormone (GH). These factors modulate the milk synthesis in lactating mothers and their disturbance can cause some problems of breastfeeding such as the agalactia or the

hypogalactia (Baig and Bhagwat, 2009; Zuppa *et al*., 2010). Some galactogogues are used to initiate, increase and maintain breast milk production. Among these drugs, metoclopramide is widely used, it antagonizes the release of dopamine in the central nervous system and increase the prolactin levels. However, these synthetic drugs present some side effects in mother and infants (Zuppa *et al*., 2010).

Milk production was slightly higher in aqueous extract treated group than blank control group. In addition, increase of milk yield was marked 23 h after the administration. Milk production depends on the number of mammary epithelial cells in the gland and their secretory activity (Capuco *et al.* 2003; Boutinaud *et al*., 2004). The increase of number or activity of mammary secretory cells are regulated by the endocrine system and the physiological state (Akers, 1985; Boutinaud *et al*., 2004). In our study the increase of milk production in lactating rats may be explained by the proliferation of mammary epithelial cells and their activity. This proliferation of mammary epithelial cells is due to the action of extract on mammary glands and some hormones involving in lactation like PRL (Lompo-Ouedraogo *et al*., 2004). In addition, Flint and Gardner, 1994 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. Our results are similar to those of Sawadogo (1987) who showed that the extract of *E. hirta* boosts and maintains the lactation in rabbits. Thereafter he found that the extract is able to induce casein synthesis in rat and stimulate prolactin secretion in ewes.

There is a linear increase of body weight of suckling pups during the period of experimentation. The variation of body weight gain of pups was observed for all groups from day 4 to day 17 of lactation. The body weight gain of pups of extract treated lactating rats and treated metoclopramide rats was slightly higher than that of the blank control group. Thereby, the pup's weight gain per day was assumed to be approximately proportional to the production of milk during the lactation. Thus, it was used as an indicator of milk production in rats (Azizah *et al*., 2012).

Conclusion:-

E. hirta contains several phytochemical compounds including steroids and triterpenoids, tannins, flavonoids, coumarins, reducing sugars which may explain its utilization in traditional medicine. Toxicity studies in rats revealed that the aqueous extract of the plant is practically non-toxic and safe for use when it administered orally. Moreover the plant exhibited a strong antioxidant activity. The aqueous extract stimulated milk production in rats, this result confirms the traditional use of the plant in the lactation insufficiency. Subsequent studies must be carried out to evaluate the effects of the plant on mouse mammary cells lines.

Acknowledgement:-

We thank Dr. Horlait Philippe (ULB, Belgium) for providing (metoclopramide) a reference molecule for the evaluation of lactogenic activity.
We are grateful to Mr. Sawadogo Stanislas for technical support during the heamatological and biochemical analysis in the "Centre médical urbain de Samadin"

References:-

1. Akers, R. M. (1985): Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. *J. Dairy Sci*., **68:501-519**
2. Amarowicz; R., Naczk, M., Shahidi, F. (2000): antioxidant activity of crude tannins of Canola and Rapeseed Hhulls. *J. A. O. C. S*., **77(9): 957-961**.
3. Azizah, M., Muhammad, N.O., Nurziana, N. (2012): Galactagogue effects of *Musa x paradisiaca* flower extract on lactating rats. *Asian Pacific J. Trop. Med.*, **882-886.**
4. Baig, M. I., Bhagwat, V. G. (2009): Study the efficacy of galactin vet bolus on milk yield in dairy cows. *Vet. World*, **2 (4): 140-142.**
5. Bakr, R. O., El-Raey, M. Abd El A., Farouk, C. N., Ahmed, H.E., Said, O. M. E. (2012): Radical scavenging activity and cytotoxicity of *Euphorbia hirta* L. growing in egypt. *Int. J. Phytomed.,* **4: 525-530**.
6. Basma, A. A., Zuraini, Z., Lacimanan, Y. L., Sreenivasan, S. (2011): Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. *Asian Pacific J. Trop. Med.,* **386-390**.
7. Boutinaud, M., Guinard-Flament, J., Jammes, H. (2004): The number and activity of mammary epithelial cells determining factors for milk production. *Reprod. Nutr. Dev*., **44: 499-508**

8. Castillo, C., Pereira, V., Abuelo, A., Hernández, J., (2013): Effect of supplementation with antioxidants on the quality of bovine milk and meat production. *Sci. World J.*, **1-8.**
9. Capuco, A. V., Ellis, S. E., Hale, S. A., Long E., Erdman, R. A., Zhao, X., Paape, M. J. (2003): Lactation persistency: Insights from mammary cell proliferation studies. *J. Anim. Sci*., 81(3):18-31
10. Ciulei, I. (1982): Methodology for analysis of vegetable drug. Practical manual on industrial utilization of medicinal and aromatic plants, Bucharest, Ministry of Chemical industry, **67 p**.
11. Colin Binns, M. B. B. S., MiKyung Lee, M. A., Wah Yun Low (2016): The long-term public health benefits of breastfeeding. *Asia-Pacific J. Public Health*, **28 (1): 7-14.**
12. Danforth, K. N., Tworoger, S. S., Hecht, J. L., Rosner, B. A., Colditz, G. A., Hankinson, S. E. (2007): Breastfeeding and risk of ovarian cancer in two prospective cohorts. *Canc. Caus. Control*, **18: 517-523**.
13. Diallo, A., Eklu-Gadegkeku, K., Agbonon, A., Aklikokou, K., Creppy, E. E., Gbeassor, M. (2010): Acute and sub-chronic (28-day) oral toxicity studies of hydroalcohol leaf extract of *Ageratum conyzoides* L (Asteraceae). *Trop. J. Pharm.Res.*, **9 (5): 463-467**.
14. Deleke-koko, I. K. E., Djego, J., Gbenou, J., Hounzangbe-Adote, S. M., Sinsin, B. (2011). Etude phytochimique des principales plantes galactogènes et emménagogues utilisées dans les terroirs riverains de la zone cynégétique de la Pendjari, *Int. J. Biol. Chem. Sci*., **5 (2): 618-633**.
15. Doukouré, M., Bayala,, B., Tindano, B., Belemtougri, G. R., Tamboura, H. H., Nwabo Kamdje, A. H., Sawadogo, L. (2018): Ethnobotanical Survey and Biological Activities of Two Lactogenic Plants in the Cascades Region of Burkina Faso. *J. Dis. Med. Pl.*. **4 (1): 1-8.**
16. Dubey, S., Mehta, S. C. (2012): Hepatoprotective activity of *Euphorbia hirta* Linn plant against carbon tetrachloride -induced hepatic injury in rats. *J. Pharm. Sci. Inn.*, **1 (3): 98-101.**
17. Flint, D. J., Gardner, M., (1994): Evidence that growth hormone stimulates milk synthesis by direct action on the mammary gland and that prolactin exerts effects on milk secretion by maintenance of mammary deoxyribonucleic acid content and tight junction status. *Endo*., **35 (3): 1119-1124**.
18. Gatti, L. (2008): Maternal perceptions of insufficient milk supply in breastfeeding. *J Nurs Scholarsh*, **40 (4): 355-363**.
19. Guenné, S., Hilou, A., Meda, R. N. T., Ouattara, N., Kiendrebéogo, M., Millogo, J. F., Nacoulma, O. G. (2012): Screening of antioxidant, anti-acetylcholinesterase and antifungal activities and HPLC-MS identification of the bioactive phenolics of *Eclipta alba* (L.) Hassk. *Int. J. Phytomed*., **4 (4): 469-476.**
20. Hinneburg, I., Dorman, H. J. D., Hiltunen, R. (2006): Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.*, **97: 122-129**.
21. Hodge, H.C., Sterner, J.H. (1943). Determination of substance acute toxicity by LD 50 . *Am. Ind. Hyg. Assoc*., 10: 93
22. Jaouad, E. H., Zafar, H. I., Badiâa, L. (2004): Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. *J. Ethnopharm.*, **91: 43-50.**
23. Kandalkar, A., Patel, A., Darade, S., Baviskar, D. (2010): Free radical scavenging activity of *Euphorbia hirta* L. leaves and isolation of active flavonoid myricitrin. *Asian J. Pharm. Clinic. Res*, **3 (3): 234-237**.
24. Kahkeshani, N., Hadjiakhoondi, A., Maafi, N., Khanavi, M. (2015): Standardization of a galactogogue herbal mixture based on its total phenol and flavonol contents and antioxidant activity. *Res. J. Pharm.*, **2 (1): 35-39.**
25. Kluwe, W. M. (1981): Renal function tests as indicators of kidney injury in subacute toxicity studies. *Toxicol. App. Pharm.*, **57: 414-424**.
26. Lanhers, M.-C., Nicolas, J.-P., Fleurentin, J., Weniger, B. (2005): Monographie de *Euphorbia Hirta* L. *Ethnopharma*., **36: 9-23**
27. Lanhers, M.-C., Fleurentin, J., Cabalion, P., Rolland, A., Dorfman, P., Misslinb, R., Peltan, J.-M. (1990): Behavioral effects of *Euphorbia hirta* L. sedative and anxiolytic properties. *J. Ethnopharm.*, **29: 189-198.**
28. Lompo-Ouedraogo, Z., Heide Daan van der, Beek E. M van der, Swarts Hans, J M., Mattheij, J. A. M., Sawadogo, L. (2004). Effect of aqueous extract of *Acacia nilotica var adansonii* on milk production and prolactin release in the rat. *J. Endocrinol.*, **182: 257-266**.
29. Majewska, M., Skrzycki, M., Podsiad, M., Czeczot, H. (2011): Evaluation of antioxidant potential of flavonoids: an *in vitro* study. *Acta Poloniae Pharmaceut. Drug Res*., **68 (4): 611-615**
30. Mohajeri, D., Mousavi, G., Mesgari, M., Doustar, Y., Nouri, M. H. K. (2007): Subacute toxicity of *Crocus sativus* L. (Saffron) stigma ethanolic extract in rats. *American J. Pharm. Toxicol.*, **2 (4): 189-193.**
31. OECD (2008): Lignes Directrices de L'OCDE pour les essais de produits chimiques: étude de toxicité orale à dose répétée pendant 28 jours sur les rongeurs**. 407: 14p**.
32. OECD (2001): Lignes Directrices de L'OCDE pour les essais de produits chimiques : Toxicité orale aiguë - Méthode de la dose prédéterminée. **420: 15p**.

33. Okamura, H., Mimura, A., Yakou, Y., Niwano, M., Takahara, Y. (1993). Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. *Phytochem.,* 33(3): 557-56.
34. Ohkawa, H., Ohishi, N., Yagi, K. (1979): Assay for lipid peroxides in animal tissues thiobarbituric acid reaction. *Analytic.l Biochem.,* **95: 351-358**.
35. Olorunnisola, O. S., Bradley, G., Afolayan, A. J. (2012): Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghia violacea* rhizomes in wistar rats . *African J. Biotechnol.,* **11(83): 14934-14940.**
36. Ouédraogo, M., Zerbo, P., Konaté, K., Barro, N., Sawadogo, L. L. (2013): Effect of long-term use of *Sida rhombifolia* L. extract on haemato-biochemical parameters of experimental animals. *Br. J. Pharmacol. Toxicol*., **4(1): 18-24**
37. Ping, K. Y., Darah, I., Chen, Y., Sreeramanan, S., Sasidharan, S. (2013): Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. *BioMed. Res. Int.*, **1-14.**
38. Qaisar, M., NaeemuddinGilani, S., Farooq, S., Rauf, A., Naz, R., Shaista, Perveez, S. (2012): Preliminary comparative phytochemical screening of *Euphorbia* species. *American-Eurasian J. Agric. Environ. Sci.*, **12 (8): 1056-1060.**
39. Sultana, A. Rahman, K. U., MS, M. S. (2013): Clinical update and treatment of lactation insufficiency. *Med. J. Islamic World Academy Sci.*, **21 (1): 19-28.**
40. Sjolin, S., Hofvander, Y., Hillervik, C. (1977): Factors related to early termination of brestfeeding. *Acta. Paediatr. Scand.*, **66: 505–511.**
41. Sawadogo, L. (1987): Contribution à l'étude des plantes médicinales et de la pharmacopée traditionnelle africaine cas des plantes lactogènes, Thèse d'Etat, **172p**.
42. Shih, M. F., Cherng, J. Y. (2006): Potential applications of *Euphorbia hirta* in pharmacology. *Drug Discovery Res. Pharm.*, **8:166-180**
43. Tiwari, P., Kumar, K., Pandey, A. K, Pandey, A., Sahu, P.K. (2011): Antihepatotoxic activity of *Euphorbia hirta* and by using the combination of *Euphorbia hirta* and *Boerhaavia diffusa* extracts on some experimental models of liver injury in rats. *Int. J. Inno. Pharm. Res*., **2(2): 126-130**.
44. Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., Rollins, N. C. (2016): Breastfeeding in the 21st Century : Epidemiology, mechanisms and lifelong effect. *Lancet* **387: 475-490**
45. Vyas, N., Argal, A. (2012): Nephroprotective effect of ethanolic extract of roots and oleanolic acid isolated from roots of Lantana camara. *Int. J. Pharmacol. Clin. Sci*., **1:54-60**
46. Wasan, K. M., Najafi, S., Wong, J., Kwong, M. (2001): Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administrationof a water soluble phytostanol compound, FM-VP4 to germils. *J. Pharm. Pharmaceut. Sci*., **4(3): 228-234**
47. Weiss, W. P. (2005): Antioxidant nutrients, cow health, and milk quality. *Penn State Dairy Cattle Nutrition Workshop*. **11-18**
48. WHO (2009): Infant and young child feeding model chapter for textbooks for medical student and allied health professionals. Session 1.
49. Yang, F. L., Li, X. S. (2015): Role of antioxidant vitamins and trace elements in mastitis in dairy cows. *J. Adv. Vet. Anim. Res.,* **2 (1): 1-9.**
50. Zimmermann, M. (1983). Ethical Guidelines for investigations of experimental pain in Conscious Animals. **16: 109-l10.**
51. Zuppa, A. A., Sindico, P., Orchi, C., Carducci, C., Cardiello, V., Romagnoli, C., Catenazzi, P. (2010): Safety and efficacy of galactogogues: substances that induce, maintain and increase breast milk production. *J. Pharm. Pharmaceut. Sci*., **13(2): 162-174**.