Keugni BA. *et al.* / *International Journal of Phytopharmacology.* 7(4), 2016, 167-177.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTI-ARTHRITIC, ANTI-GRANULOMATOUS AND ANTI-PYRETIC EFFECTS OF *CYLICODISCUS GABUNENSIS* ETHYL ACETATE EXTRACT (HARMS) IN RATS

BA Keugn[1], F. Longo[2*], PD. Dzeufiet Djomeni[1], S. Fogue Kouam[3], M. Spiteller[4], T. Dimo[1], LS. Etoundi Ngoa[2]

[1]Department of Animal Biology and Physiology, University of Yaoundé I, P.O Box 812, Yaoundé Cameroon.
[*2]Laboratory of animal physiology, Department of Biological Sciences, Higher Teacher's Training College, University of Yaoundé I, P.O Box 47 Yaoundé Cameroon.
[3]Department of Chemistry, Higher Teachers Training College, University of Yaoundé I, P.O. Box 47, Yaoundé I, Cameroon.
[4]Institute of Environmental Research (INFU) of the Faculty of Chemistry TU Dortmund, Otto-Hahn-Str. 6, D-44221, Dortmund, Germany.

ABSTRACT

*Cylicodiscus gabunen*sis is used in traditional medicine for the treatment of headache, rheumatism, malaria and inflammatory related diseases. This work aimed to evaluate the anti-arthritic, anti-granulomatous and anti-pyretic effects of the ethyl acetate extract of *Cylicodiscus gabunensis* (EACg) in rats. The arthritis was induced with the Complete Freund Adjuvant, while the granuloma formation was induced by a carrageenan air pouch. Fever was induced by a brewer's yeast suspension. A High Performance Liquid Chromatography (HPLC) was done for the extract active compounds analysis .The results showed that EACg (200 and 400mg/kg) and dexamethasone (1mg/kg), administered per os significantly reduced the paw oedema, SGOT, SGPT, cholesterol, triglycerides, LDL, nitrites, MDA level, and increased the levels of HDL, glutathione, catalase and SOD. The dose 200mg/kg reduce the granuloma tissue weight, ($p<0.01$) the volume of the exudate ($p<0.01$) and the migration of the white blood cells into the exudate ($p<0.01$), all the same as indomethacin (3mg/kg *p.o*).The fever was also reduced ($p<0.01$) by EACg and aspirin. The EACg anti-arthritic, anti-granulomatous and anti-pyretic, properties might be due to the presence of compounds such as cylicodiscoside, gabunoside and cyclodione revealed by the HPLC.

Key words: Arthritic; Granuloma; Leukocytes, Oedema; Pyretic; Complete Freund Adjuvant.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease, which is characterized by chronic inflammation of the synovial tissues in multiple joints. RA can lead to joint destruction through inflammatory involvement of the synovial membrane, cartilage, and subchondral bone (Ramesh *et al*. 2013).

Inflammatory process has two phases: acute and chronic. Acute inflammation is characterized by fever, pain, and oedema, while chronic inflammation is characterized by cellular proliferation and granuloma formation **(**Rousselet *et al.*, 2005). Pyrexia is the body's natural defence to create an environment where infectious agent or damaged tissue can't survive (Cheng *et al*., 2005). Elaboration of interleukin-1 and tumour necrosis factor-α is believed to initiate the synthesis and release of the fever-causing autacoid prostaglandin E2 (PGE_2) by the endothelium and pericytes of brain capillaries (Steiner *et al*., 2006).

Corresponding Author

Longo Frida
Email: longofri@yahoo.fr

In clinic, there are various drugs such as azathioprine, tumor necrosis factor alpha (TNFα) blockers, non-steroidal anti-inflammatory drugs (NSAIDs) that are used for the treatment of RA. Although these drugs can temporarily alleviate the symptoms, they do not halt progression of joint destruction and they are accompanied with many undesirable adverse effects, including gastrointestinal bleeding, renal or hepatic failure (Gege-Adebayo and Shafe, 2013). This makes these drugs widely unacceptable, especially in the elderly where the disease is more prevalent (Osadebe and Okeyé, 2003). Furthermore, the rising costs of orthodox medicine and the scarcity of some drugs give the phytomedicinal treatment a very important place in the management of inflammatory diseases. Among plants used against fever and inflammatory diseases, *Cylicodiscus gabunensis* (Mimosaceae*)* known as Adoum bokoka, by Eton population of Cameroon, is a very big tree that grows mainly in damp equatorial forest of Central and West Africa (Adjanohoun, 1996). The stem bark extract of this plant is used in traditional medicine as remedies for head ache, rheumatism, diarrhoea, (Kouitcheu *et al.*, 2006) and malaria (Okokon *et al*., 2002). The Ethyl acetate extract of the stem bark of *Cylicodiscus gabunensis* showed antimicrobial activity against pathogenic species isolated from patients including *Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, and Morganella morganii* (Kouitcheu and Etoa, 2007).

The ethyl acetate fraction this plant has analgesic and anti-inflammatory properties (Keugni *et al*., 2014). Considering the traditional uses of *Cylicodiscus gabunensis* stem's barks, the present study was undertaken to investigate its anti-arthritic, anti-pyretic and anti-granulomatous properties in rats.

METHODOLOGY

Collection and identification of the plant material

The stem barks of *Cylicodiscus gabunensis* were harvested in April 2012, at Mbankomo (Center Region Cameroon), and a voucher specimen was deposited at the National Herbarium of Yaoundé, under the number 2154/ SRF/ Cam, after botanical identification by Dr Nole, a plant taxonomist in the Medical Institute of plants and Medicinal research (IMPM) of Yaoundé.

Preparation of plant extract

The powder (2500g) obtained from the air dried and grounded stem barks of *C. gabunensis* was macerated during 72 hours in 6L of methylene chloride/methanol (1:1). After filtration using a Whatman paper N^{o}3, filtrate was concentrated using a HEIDOPHW 2000 rotary evaporator at 40 °C to obtain 197.67 g (yield 7.91 %) of a brown powder. Thereafter, 100g of this dough was exhausted in 1.5L of ethyl acetate, and concentrated using a rotary evaporator, giving 38g (yield 38 %) of a brown ethyl acetate fraction of the methyl chloride methanol.

High Performance Liquid Chromatography of the EACg

The High Performance Liquid Chromatography (HPLC) of the EACg was carried out according to the protocol described by Longo *et al*., (2015).

Animals

Adults Wistar males and females rats weighting 150g to 180g were used for this work. Animals were bred in the animal house of the Faculty of Science, University of Yaoundé I, in natural light conditions (12 light and 12 dark cycles). All the animals were allowed to have free access to food (standard diet for rodent) and water. Animals were deprived of food 14 hours, but allowed free access to water before the experiment. All experiments were performed according to guidelines for the care of laboratory animals from the Cameroon National Ethical Committee (Ref. no Fw-IRB00001954).

Drugs and chemicals

The drugs used for the experimentation were obtained from the pharmaceutical factory Sigma Aldrich, except valium and ketamine obtained from the pharmaceutical factory Roche. Chemicals used were obtained from local institute store.

Experimental procedure

Freud's Complete Adjuvant (FCA) induced arthritis in rat

Twenty female's rats were used for arthritis induction. Arthritis was induced by a single right hind paw injection of 0.1mL of Freund's complete adjuvant (FCA). The swelling paw was measured up to 21 days using a plethysmometer (Ugo Basile, Italy, model 7140) at the moment 0h; 2h; 4h; 24h; then 5 ; 9 ;13; 17 ; and 21days (Suha, 2011). On the day 9, animals were divided into six groups and received treatments *via* oral route, once per day, up to the days 21 as followed:

Group 1: Control non arthritic rats received DMSO 2%.
Group 2: Arthritis induced rats received DMSO 2 %.
Group 3: Arthritis induced rats received dexamethasone 1mg/kg.
Group 4: Arthritis induced rats received EACg 200mg/kg.
Group 5: Arthritis induced rats received EACg 400mg/kg.

On the day 21, all rats were anesthetized by intraperitoneal injection of valium (2mg/kg) and secondary ketamine (1mg/kg), then scarified by carotid section. The blood was collected in dry tubes for determination of the liver function such as Serum Glutamate Oxaloacetate Transferase (SGOT) and Serum Glutamate Pyruvate Transferase (SGPT) and lipid metabolism (HDL; LDL; triglycerides; cholesterol). The liver and spleen were removed, homogenized in Tris-HCl

buffer (0.1M, pH7.4) for the glutathione, catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and nitrite contain determination.

Carragenan air pouch induced granuloma

The protocol used in this test was described by Dajeong *et al.*, (2012), with slight modifications. Rats were divided into 5 groups of 5 females' rats each. The animals of the first four groups were shaved on their back using a sterilised scissors, after anaesthesia with valium (3mg/kg) and ketamine (10mg/kg) through intraperitoneal pathway. The fifth unshaved remaining group was used as the control group, and they received neither air nor carrageenan. Thereafter, the shaved zone was disinfected with alcohol 70% using a sterilised syringe of 10mL, a pouch of 6mL of air was created in the shaved back, and filled with 4 mL of a saline NaCl (0.9%) carrageenan solution 2%. After injection with carrageenan, animals were treated during seven days by oral route as follow: The control group and one of the shaved group received DMSO 2%, 1mL per 100g *b.w*. Two other groups received the plant extract at the doses of 200 and 400mg.kg^{-1} respectively. The last group received indomethacin 3mg/kg. On the day 8, animals of all groups were sacrificed under slight anaesthesia with ether. The blood was collected in EDTA tubes for white blood cells counting. Thereafter, the accumulated exudate in each dorsal pouch was collected by puncture using a 10mL sterile syringe. The volume of the exudate was measured, the number of white blood cells in the exudate was counted using a Malassez cell with 1/10 dilution factor.

The blood leukocytes counting, was performed by an automatic counter (Sysmex-poch 100i). Each blood sample collected in EDTA tube was introduced in the cell counter. The number of lymphocytes, monocytes, granulocytes and the total leukocytes was obtained directly from the counter on a printed sheet of paper. For each animal, the granuloma tissue was carefully removed, then dried during 48 hours in the oven at 60 °C, and weighed with an accurate scale.

The percentages of inhibition relative to the exudate volume, granuloma's weight, or white blood cells number in the exudate were calculated using the formula:

$P = [1-(X_{treated}/X_{negative\ control})] \times 100$:(*X= mean value of the parameter concerned).*

The number of white blood cells was converted after the Malassez cell counting using the formula**:**

$$\mathbf{Q}=\frac{Nxfdx10^6}{nxV}$$ ***N:** number of white blood cell counted with Malassez cell*

***V:** volume of one rectangle of Malassez cell (0.01 mm³)*

***fd:** dilution factor = 1/10 ;* ***n** = 4 (number of rectangle in which cells were counted)*

***Q**: Number of leukocytes per litre of exudate*

Yeast induced pyrexia

The antipyretic activity of the plant extract was assessed by the method described by Parimalakrishnan *et al.,* **(**2007). Male Wistar rats were fasted overnight with water *ad libidium* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (W/V) brewer's yeast suspension (10mL/kg) into the rat's neck region. Eighteen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato Keiryoki Mfg. Co., Ltd. Japan). Only rats that showed an increase in temperature in the range 0.5-1.5°C were used for experiments and divided in four groups of five rats each. The extract 200 and 400mg/kg the DMSO 2%, and aspirin 150mg/kg was administered orally and the temperature was measured at 0.5; 1; 2; 3; 4; 5; and 6 hours after drugs administration.

Statistical analysis

Each result was presented as mean ± S E M (Standard Error on Mean). One way ANOVA followed by Turkey tests were performed, and the P value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Effects of EACg on the paw volume

Injections of FCA to rats induced a paw oedema of 0.4 ± 0.03mL nine days later (Fig 1). From the day 9, administration of dexamethasone and EACg significantly reduced the oedema by 71.74 % ($p<0.001$); 53.91% ($p<0.01$); 45.21% ($p<0.01$) on the day 21, respectively for dexamethasone, the doses 200 and 400mg/kg of EACg.

Effects of EACg on some serum biochemical parameters

FCA during 21 days significantly ($P<0.05$) increases in the negative control group, when compared to the control, the liver SGOT and SGPT by 263.75% and 119.82% respectively (Tab 1). Administration of EACg and dexamethasone significantly reduced these transaminases rate. For the EACg (200mg/kg), the percentage of reduction was 311.65% and 59.48% for the SGOT and SGPT respectively compared to 277.83% and 136.89 % for dexamethasone. FCA in the negative control group also significantly increased the serum concentration of cholesterol (61.03%), LDL (520.69%), triglyceride (115.17%), and decreased the concentration of HDL (120.80%). Administration of EACg (200 and 400mg/kg) significantly reduced the cholesterol level (81.77%; 66.71%), LDL (690.00 %; 197.27%), triglyceride (100.37%; 61.36%) and increased the level of HDL (88.77%; 66.71%). Dexamethasone also significantly reduced the level of cholesterol (72.09%), LDL (493.87%); Triglyceride (116.19%) and increased the level of HDL (102.83%).

Effects of EACg on some tissue biochemical parameters

The dose of 200mg/kg of EACg reduced respectively in the liver and spleen, the amount of proteins (97.95%; 69.45%), MDA (262.49%; 427.30 %) and nitrites (64.40%; 140.12%), compared to 92.69%; 191, 90%; 74.39% for dexamethasone in the liver and 66.67%; 403.79%; 180.63% in the spleen respectively for proteins, MDA and nitrites. In contrast, on the negative control, FCA, significantly ($p<0.001$) increased proteins (119.40%; 68.04 %), malondialdehyde ($P<0.001$), MDA (269.51%; 471.27%) and nitrites (80.54 %; 198.86 %) levels on the liver and spleen, (Fig 2_{A-B-C}).

The EACg reduced the concentration of glutathione GLU (114.83%; 206.41%) and the activity of catalase, CAT (341.92%; 295.07%) and superoxide dismutase, SOD (234.06%; 119.32%) The EACg at the dose of 200mg/kg as well as dexamethasone was efficient than the dose 400mg/kg to improve the parameters of oxidative stress. However, the EACg (200mg/kg) significantly ($P<0.001$), increased in the liver and the spleen respectively, the activity of CAT (335.51%, 292.08%); SOD (194.26%; 122.70%) and the concentration of GLU (135.51%; 201.52%) (Fig $2_{D-E-,F}$).

Effect of EACg on granuloma tissue weight in rat

The administration of the ethyl acetate extract of *Cylicodiscus gabunensis* at the doses of 200 and 400mg.kg^{-1} significantly ($p<0.01$) reduced the weight of the granuloma tissue in rat (Fig.3). In the negative control group, the carrageenan induced the formation of 4.20 ± 0.22g of granuloma tissue. In the presence of the plant extract at the doses of 200 and 400mg/kg, the weight was reduced to 2.23±0.13 ($p<0.001$) and 3.24±0.05 g ($p<0.01$) respectively, corresponding to 46.63%, and 22.69% respectively. Indomethacin also displayed a significant reduction of the granuloma weight up to 53.33% ($p<0.001$).

Effects of EACg on the exudate volume

The EACg reduced exudate accumulation in the back air pouch, with a significant percentage of inhibition of 56.63 % at the dose of 200mg/kg ($p<0.001$), compared to 58.41% inhibition for indomethacin ($p<0.001$). In the negative control group, the carrageenan induced the accumulation of 8.32 ± 0.22mL of exudate (Fig 4).

Effects of EACg on the number of white blood cell in the exudate and in the blood

Injection of carrageenan in the rat back pouch induced accumulation of white blood cells in the exudate (Fig 5_A). In the blood, the number of total white blood cells in the negative control group was significantly ($p<0.001$) decreased *vs* the control (Fig 5_B). The EACg at all the doses, significantly reduced the number of white blood cells in the exudate, and increased their number in the blood. At the dose of 200mg/kg, the number of total white blood cells was increased from 7.34 ± 1.34 x10^3 (negative control) to 17.40 ± 3.31 x10^3 per micro litre of blood ($p<0.001$). At this same dose of 200mg/kg, the number of blood leukocytes was 4.99 ±0.28 x10^3; 9.96 ± 1.4 x10^3 ; 3.67 ± 0.29 x10^3 per µL of blood, respectively for granulocytes (Figure 5_C); lymphocytes (Fig 5_D) and monocytes (Fig 5_E). Indomethacin also significantly increased the number of polymorphonuclear cells (4.18 ± 0.30 x10^3), lymphocytes (6.92 ± 1.10 x10^3) and monocytes (3.02 ± 0.36 x10^3).

Effect of the EACg on the yeast induced pyrexia in rat

All the doses of the extract showed a significant antipyretic activity similarly to that of the standard drug aspirin ($p < 0.001$). The subcutaneous injection of yeast suspension marked elevated rectal temperature 18 hours after injection (Tab 2).

High Performance Liquid Chromatography of the EACg

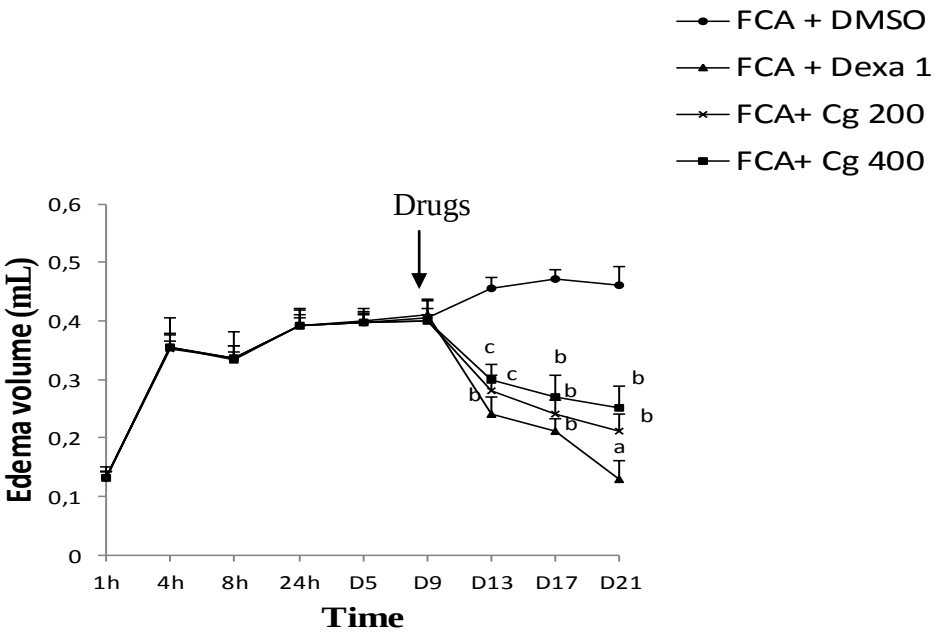
The chromatogram of the ethyl acetate extract of *Cylicodiscus gabunensis* stem bark showed the relative abundance of different compounds (Fig 6). The study of the chromatogram permitted to identify tree triterpernoids identified as gabunoside, cylicodiscoside and cyclodione.

Table 1. Effects of EACg on Freud's Complete Adjuvant induced some serum biochemical parameters

	Control	***FCA+DMSO***	***FCA+Dexa***	***FCA+Cg200***	***FCA+Cg400***
TG (mg/dL)	54.10±5.52	***116.41±9.20^d***	***53.84±4.67^a***	***57.43±6.50^a***	***72.05±5.75^a***
HDL (mg/dL)	116.47±7.54	***51. 81±4.94^d***	103.80±8.09^a	97.80±9.89^a	***86.37±9.10^{bf}***
LDL (mg/dL)	29.67±3.21	***184.16±16.79^d***	***31.01±2.56^a***	***23.29±7.68^a***	***61.95±8.32^{bf}***
CHO (mg/dL)	***146.15±6.35***	***235.36±18.24^d***	***136.83±9.70^a***	***129.97±6.54^a***	***140.53±10.87^{bf}***
SGPT (UI)	***16.98±1.84***	***37.32±4.45^d***	***15.54±1.70^a***	***23.4.03±1.26^{bf}***	25.80±1.53^{bf}
SGOT (UI)	***30.68±1.40***	***11.58±1.90^d***	***29.51±1.11^a***	***27.09±1.60^a***	***34.43 ±4.2^a***

Values are means ± SEM, *n=5* .a=***; $p<0.001$; b=**$p<0.01$; significant differences *vs* the negative control (FCA+DMSO). d=+++$p<0.001$; f=+$p<0.05$ significant differences *vs* the normal control (DMSO). Dexa: dexamethasone; FCA: Freund's Complete Adjuvant, Cg: Cylicodiscus gabunensis, DMSO: Dimethyl sulfoxyde; EACg: Ethyl acetate extract of Cylicodiscus gabunensis.

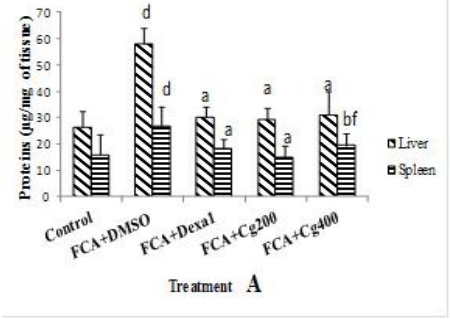
Fig 1. Inhibition of Freud's Complete Adjuvant -induced hind paw swelling by EACg

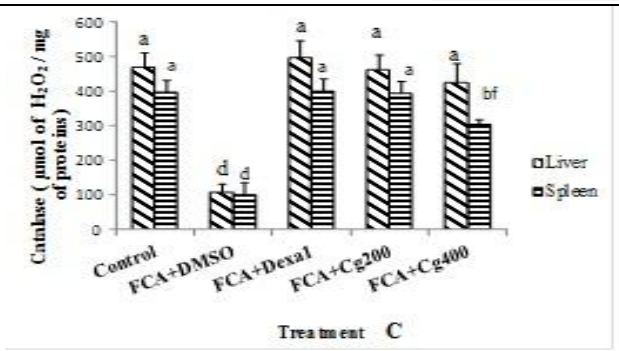


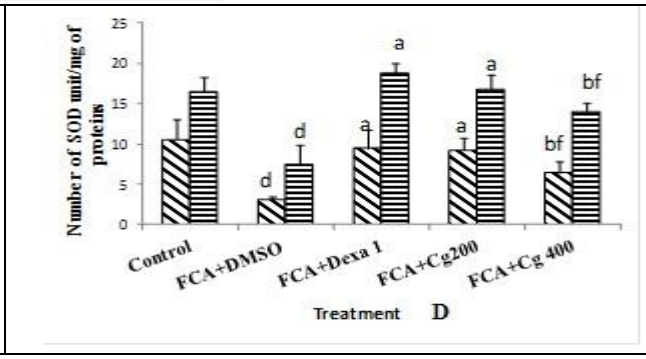
Curves represent oedema volume ± SEM (mL), *n=5*

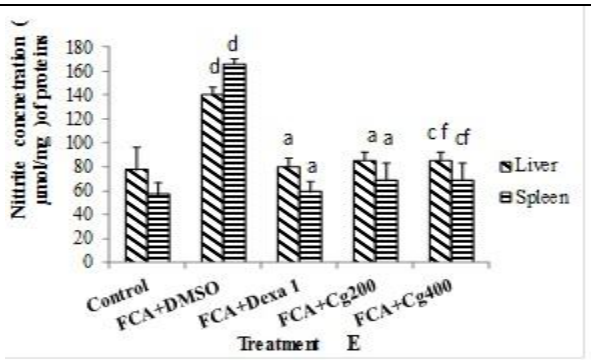
a= ***p<0.001; b= **p<0.01 ; c =*p<0.05 significant differences vs the negative control (FCA+ DMSO); Cg : *Cylicodiscus gabunensis* ; Dexa : Dexamethasone; FCA : Freund's Complete Adjuvant; DMSO : Dimethyle sulfoxyde ; EACg : ethyl acetate extract of *Cylicodiscus gabunensis*, D : day; h : hour.

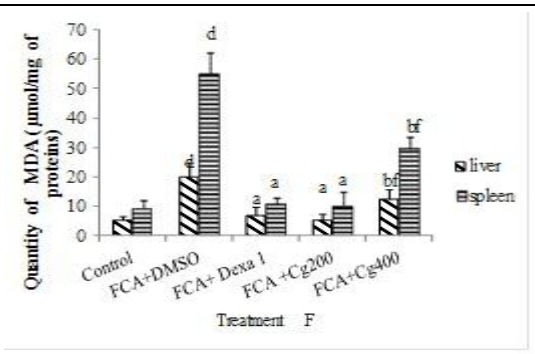
Fig 2. Effects of EACg on the concentration of proteins (A); GLU (B); CAT (C); SOD (D); nitrite (E); MDA (F)





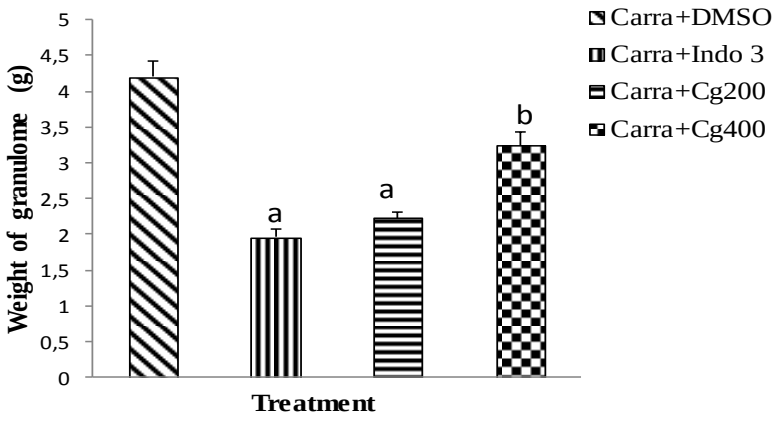






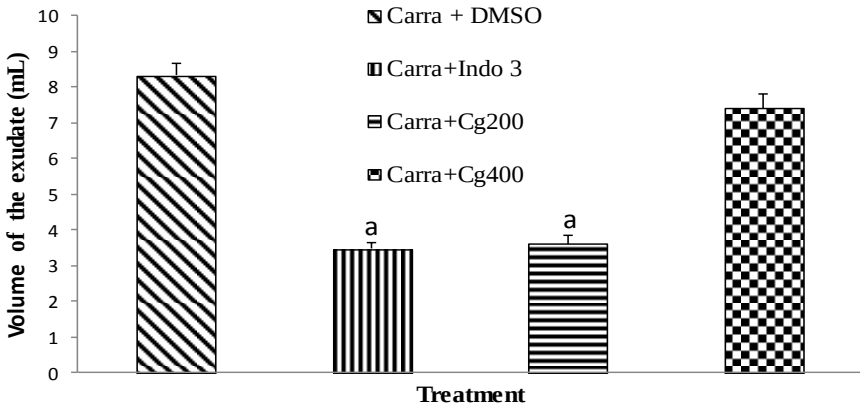
Each bar represents mean ± SEM, *n=5* a=***p<0.001; b=**p<0.01; c=*p<0.05: significant differences *vs* the negative control (FCA+DMSO). d=+++p<0.001; e=++p<0.01; f=+p<0.0: significant differences *vs* the control (DMSO). Dexa: dexamethasone, FCA: Freund's Complete Adjuvant, Cg: *Cylicodiscus gabunensis*, DMSO: Dimethyle sulfoxyde; EACg: ethyl acetate extract of *Cylicodiscus gabunensis*; MDA: Malondialdehyde, SOD: Superoxide dismutase; CAT: Catalase; GLU: Glutathione.

Fig 3. Effects of EACg on granuloma tissue weight in rat



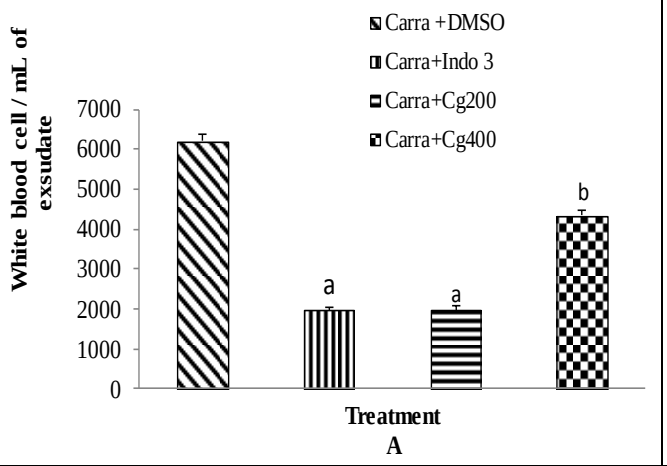
Each bar represents mean weight of granuloma tissue ± SEM (g), *n= 5* a=*** p<0.001; b=** p<0.01, values are significant when compared to the negative control group (carra +DMSO).
Carra: carrageenan; Indo: indomethacin; *Cg: Cylicodiscus gabunensis;* EACg: Ethyl acetate extract of *Cylicodiscus gabunensis*; DMSO: dimethyl sulfoxyde

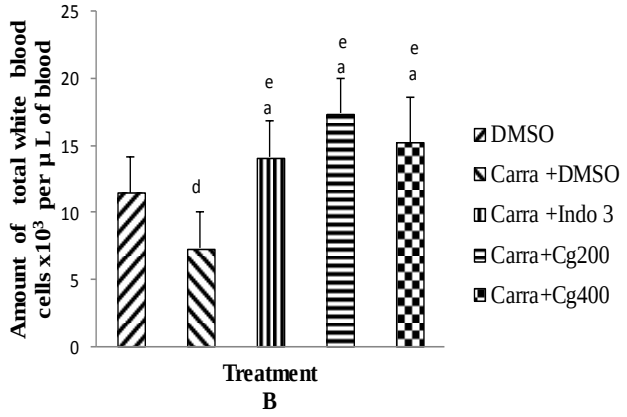
Fig 4. Effects of EACg on the exudate volume in rat

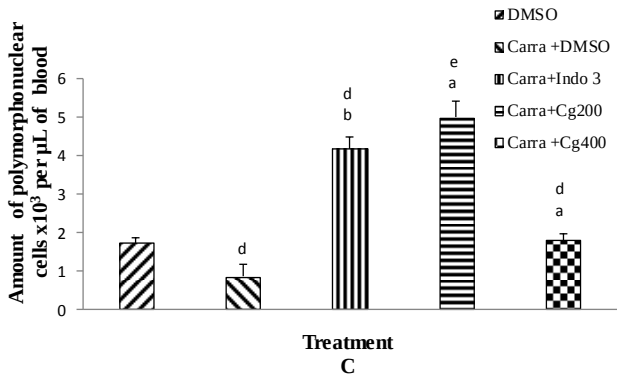


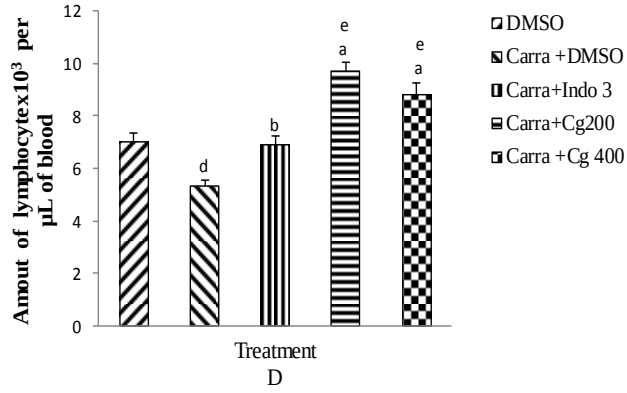
Each bar represents mean exudate volume ± SEM (mL), *n= 5*; a=***p<0.001, significant difference *vs* the negative control group (carra+DMSO). Carra: carrageenan; Indo: indomethacin; *C.g: Cylicodiscus gabunensis;* EACg: Ethyl acetate extract of *Cylicodiscus gabunensis*; DMSO: dimethyl sulfoxide.

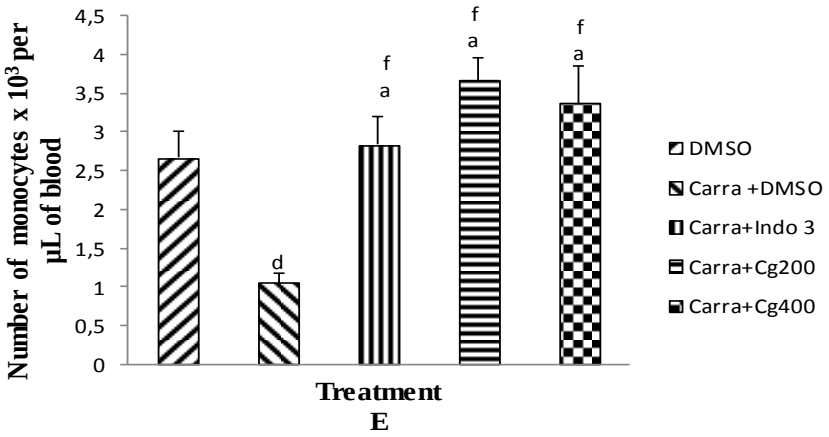
Figure 5. Effects of EACg on exudate white blood cells (A); total blood leukocytes (B); blood polymorphonuclears amount (C); blood lymphocytes amount (D); blood monocytes amount (E).











Each bar represents mean ± SEM, *n= 5*. a=***p<0.001; b=**p<0.01; c=*p<0.05: significant differences *vs* the negative control (carra+DMSO). d=+++p<0.001; e=++p<0.01; f=+p<0.0 significant differences *vs* the normal control group (Water) Carra: carrageenan; Indo: indomethacin; *Cg: Cylicodiscus gabunensis*; EACg: Ethyl acetate extract of *Cylicodiscus gabunensis* DMSO: Dimethyl sulfoxide.

Fig 6a. Spectra fingerprint HPLC of the EACg

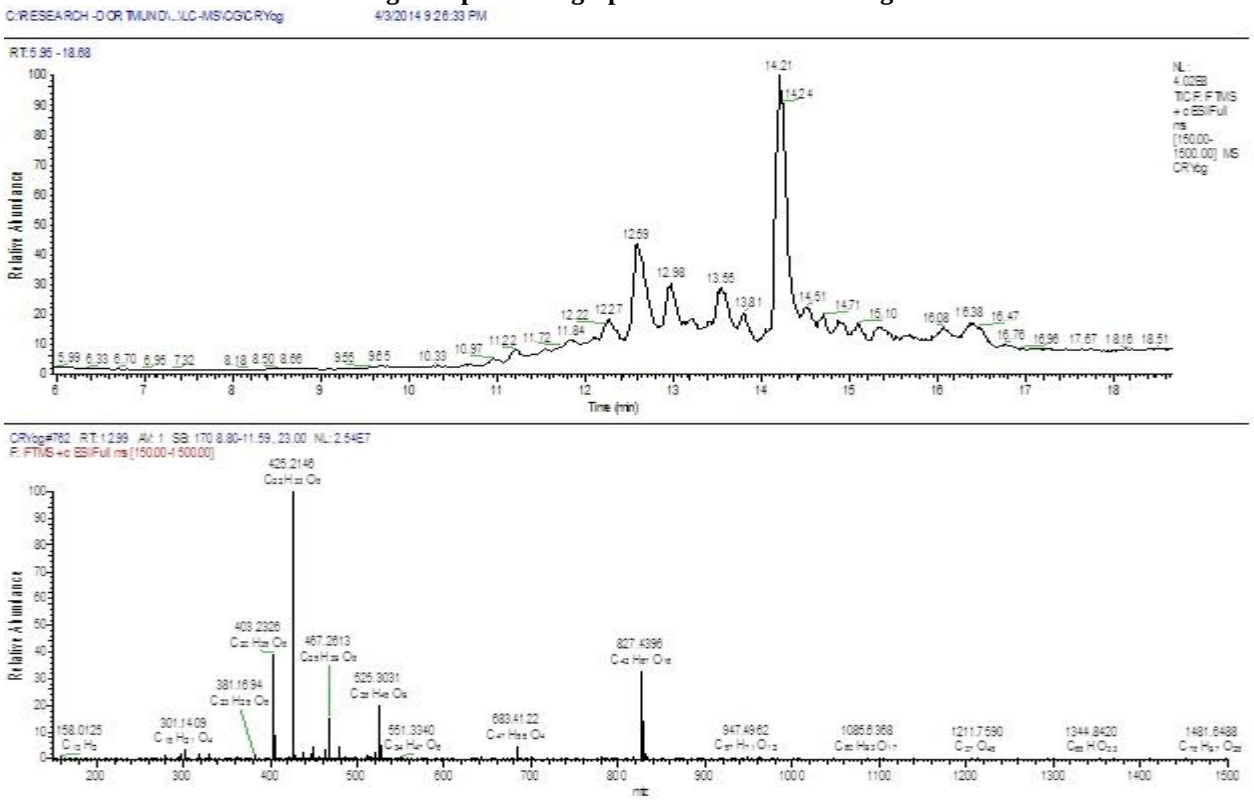
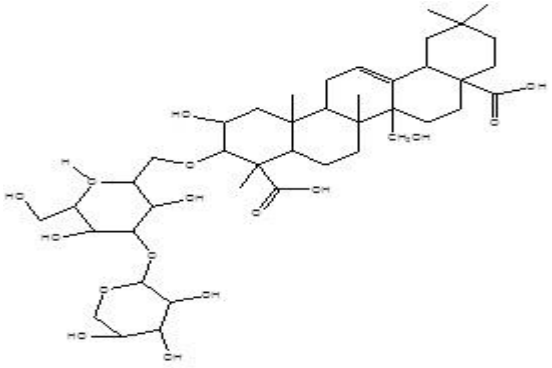
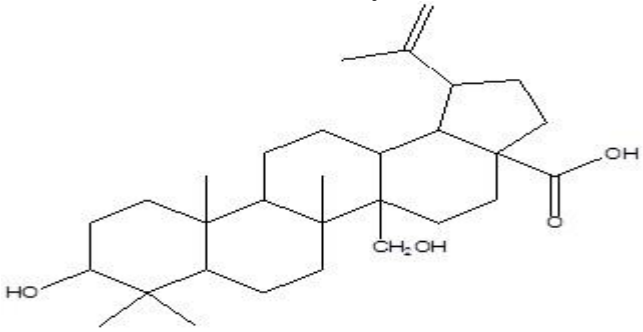


Fig 6b. Triterpenes arising from the fingerprint HPLC of EACg

[M+H]+ = 827.43965: Gabunoside.



[M+H]+=473.3622 : Cylicodiscoside



[M+H]+ = 601.4256: Cyclodione

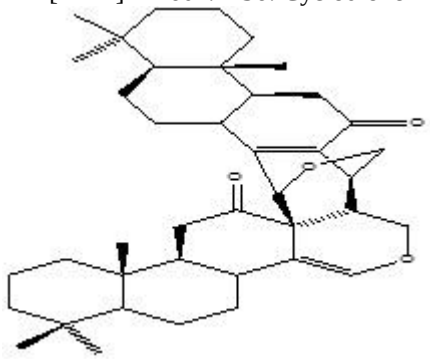


Table 2. Inhibition of the yeast produced pyrexia *by* the *EACg* in rat

Treatment Dose(mg/kg)		Average rectal temperature ± SEM(°C)								
		Tn	Ti	1/2h	1h	2h	3h	4h	5h	6h
DMSO		37.25 ±0.17	38.32 ±0.10	38.34 ±0.22	38.42 ±0.09	38.60 ±0.05	38.44 ±0.13	38.42 ±0.05	38.08 ±0.22	38.00 ±0.05
Asp	150	37.18 ±0.22	38.40 ±0.17	38.38 ±0.16	38.16 ±0.17	38.14 ±0.23[b]	37.48 ±0.22[a]	37.42 ±0.13[b]	37.34 ±0.01[b]	37.20 ±0.10[a]
EACg	200	37.33 ±0.12	38.44 ±0.13	38.60 ±0.16	38.22 ±0.13	37.52 ±0.12[a]	37.44 ±0.33[a]	37.42 ±0.08[b]	37.42 ±0.04[b]	37.40 ±0.20[b]
EACg	400	37.24 ±0.24	38.14 ±0.26	38.10 ±0.32	37.8 ±0.18[a]	37.6 ±0.15[a]	37.48 ±0.21[a]	37.34 ±0.19[b]	37.36 ±0.17b	37.32 ±0.09[b]

DISCUSSION

Arthritic animal treated with dexamethasone or the plant extract developed low oedema volume. This can be explained by the inhibition of neutrophil migration, and exudate formation by dexamethasone (Subash, 2012). Terpernes contain in the EACg may display anti-oedematous effects by the same mechanism as dexamethasone. Freund's adjuvant induced arthritis occurs through cell-mediated autoimmunity. Inoculation of FCA activates macrophages and lymphocytes or their products such as, cytokines, and chemokine which are involved in abnormal lipid and protein metabolism (Schorlemmer and Bartlett, 1999).

FCA also induced free radicals production, which lead to membrane peroxidation with MDA level increase. Furthermore, free radicals provoke depletion of glutathione concentration, and the activity of anti-oxidant enzymes as CAT and SOD in tissues like liver, spleen, and kidney (Jung *et al.*, 2005) then increase the spleen and liver contain in MDA and nitrite. The HDL, the glutathione and the activity of peroxidase (CAT, SOD) were also significantly reduced. Administration of the plant extract and dexamethasone to the rats, significantly improved the transaminases activity, the lipid metabolism, and the anti-oxidant status of rats due to the EACg to stabilize the membrane and to trap free radicals generated in RA pathology. The RA is characterized by cell migration and infiltration. The carrageenan air pouch is a good model for the study of migration of cell during sub-acute inflammation (Fattorusso and Ritter 2004). Angiogenesis, nitric oxide synthesis and kinins secretion seems to be the main factors that trigger granuloma tissue formation (Fattorusso and Ritter, 2004).

The accumulation of leukocytes in the site of inflammation especially neutrophil and macrophages induced the formation a granuloma tissue with white blood cells infiltration (Suresha *et al*., 2012). The anti-granulomatous property of the plant extract may be through the inhibition of nitric oxide and kinin synthesis, hence inhibition of fibroblast proliferation and collagen synthesis. Similar results were obtained by Shivani *et al*., (2012), who demonstrated that the herbs of *Laghupan chamul*a reduced the weight of granuloma tissue and the volume of the exudate in the oedema induced by turpentine oil. Simultaneously in the blood, great changes were noticed on the blood leukocyte number. Hyperleucocytosis observed in our study is the aftermath of white blood cells proliferation in response to the presence of a foreign phlogostic agent. These cells are recruited from the circulation by the steady release of chemotactic factors (De heras and Sonsoles, 2009). The role of cell adhesion molecules (CAMs), such as intercellular cell adhesion molecule-1 (ICAM-1), has been studied extensively in the process of inflammation. These molecules are responsible for recruiting leukocytes into the vascular endothelium before extravasation to the injured tissues (Kobayashi and Boelte, 2007). Administration of indomethacin and the plant extract to rats significantly prevent cellular migration into the exudate. In fact, indomethacin is an anti- inflammatory drug that impairs inflammatory response by many mechanisms among which inhibition of the white blood cells migration towards the inflammatory site (Suresha *et al*., 2012). The EACg may act similarly on inflammation. Prostaglandins E2, has been described as an effective mediators in the pathophysiology of RA (Jennifer *et al*., 2002).

Prostaglandins E2 induced vasodilatation and stimulate the hypothalamus to produce fever (Steiner *et al.*, 2006). Yeast is among factors that stimulate prostaglandins production to induce fever (Parimalakrishnan, 2007). The fever condition enhanced formation of cytokines such as interleukins, interferons and tumour necrosis factor α, inducing the synthesis of prostaglandin (Dajeong *et al*., 2012).

The plants extract at all the doses, and aspirin decrease the body temperature. Aspirin, a non-steroidal anti-phlogistics display antipyretic activity by inhibiting the cyclooxygenase 2, an enzyme responsible for the synthesis of prostaglandins of series E (Cheng *et al*., 2005). Some secondary metabolites of the plant extract as characterized by Keugni *et al*., (2014) and Kouitcheu *et al.,* (2006) may act by the same mechanism as aspirin.

The qualitative analysis of EACg by HPLC coupled to the mass chromatography revealed the presence of three triterpernoids previously isolated in the stem bark of *C. gabunensis* .and named as gabunoside, cylicodiscoside and cyclodione (Tane *et al*., 1995; Tene *et al*., 2010). It has been reported that triterpernoids possess various pharmacological properties including anti-inflammatory activity (Geetha, and Varalkshmi, 2001). The dose 200 mg/kg was more efficient than 400 mg/kg.

CONCLUSION

According to these results, the anti-arthritic, anti-granulomatous, and anti-pyretic properties of EACg stem bark might be due to its content in triterpernoids that interact with nitric oxide, pathways, free radical neutralization. These pharmacological activities justified the traditional use of *Cylicodiscus gabunensis* in the management of inflammatory diseases such as malaria and rheumatism.

ACKNOWLEDGMENT

We sincerely acknowledge gratitude to the head of the laboratory of Animal Physiology of the Faculty of Science of the University of Yaoundé I (Cameroon), for the equipment and reagents. We also thank, heart warmly, Misses KAMENI Mireille, MENGUE Sandrine, TCHOUPOU Hugette, and Mr NGOKO Rodrigue, all from the University of Yaoundé I for their technical support.

This work was supported in part, by the German Academic Exchange Service (DAAD) initiative ‘‘Welcome to Africa’’ and the Cameroonian High Education Ministry through the “special allocation account for the modernization of University research” in Cameroon.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Adjanohoun J, Aboubakar N, Dramane K, Ebot M, Ekpere J and Enow-Orock E. Contribution to floristic and ethnobotanic in Cameroon, medicine and traditional. Pharmacopeia. Technical Commission Research from the United African Organisation. UAO/TCR. 1996.
2. Cheng L, Ming-liang H and Lars B. Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial. *Acta Pharm Sinica*, 8(26), 2005, 926–33.
3. Dajeong K, Dongsun P, Jangbeen K, Yun-Hui Y, Ehn-Kyoung C, Yoon-Bok et al. Anti-inflammatory effects of Houttuynia cordata supercritical extract in carrageenan-air pouch inflammation model. *Lab Anim Res*, 28(2), 2012, 137–142.
4. De heras B and Sonsoles H. Molecular basis of the anti-inflammatory effects of terpenoids. *Inflamm Allergy*, 8, 2009, 28–39.
5. Fattorusso V and Ritter O. Vademecum clinique, du diagnostic au traitement. Ed Masson. Paris, 2004.
6. Geetha, and Varalkshmi. Anti-inflammatory activity of lupeol and lupeol linoleate in rats. *J Ethnopharmacol*, 76, 2001, 77–80.
7. Gege-Adebayo G, Bassi A, Igbokwe V and Shafe M. Antipyretic effect of Ocimum gratissium on brewer yeast induced fever in wistar rats. *J Med Sci*, 4(6), 2013, 247–51.
8. Jennifer M, McCoy, Joan R, Wicks and Laurent P. The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. *J Clin Invest*, 110(5), 2002, 651–658.
9. Jung HJ. Nam JH, Choi J, Lee KT. and Park HJ. Anti-inflammatory effects of chiisanoside and chiisanogenin obtained from the leaves of Acanthopanax chiisanensis in the carrageenan and Freud‘s complete adjuvant-induced rat paw edema. *J Ethnopharmacol*, 97, 2005, 359–367.
10. Keugni BA, Longo F., Fotio FA., SF Kouam, Dimo T. and Etoundi Ngoa LS. Analgesic and anti-inflammatory properties of the ethyl acetate extract of Cylicodiscus gabunensis (Mimosaceae) in rodents. *World J Pharm Pharm Sci*, 3(12), 2014, 1538–1554.
11. Kobayashi H and Boelte. Endothelial cell adhesion molecules and cancer progression. Curr Med Chem, 14(4), 2007, 377–86.
12. Kouitcheu M, Kouam J, Penlap BV, Ngadjui,TB, Fomum ZT and Etoa FX. Evaluation of Antimicrobial Activity of the Stem Bark of Cylicodiscus gabunensis (Mimosaceae). *Afr J Tradit Med*, 4(1), 2007, 87–93.
13. Kouitcheu ML., Penlap B., Kouam J., Ngadjui B., Fomum Z. and Etoa F. Evaluation of the antidiarrheic activity of the stem barks of Cylicodicus gabunensis (Mimosaceae). *J Ethno pharmacol*, 111, 2006, 597–606.
14. Longo F., Teuwa M., S. Kouam Fogue, M. Spiteller, Etoundi Ngoa L.S. Heptoprotective effect of Canna indica L. rhizome against Acetaminophen (Paracetamol). *World J Pharm Sci*, 4 (5), 2015, 1609-1624.
15. Okokon J., Ita B. and Udokpoh AE. Antiplasmodial activity of the ethanolic extract of Cylicodiscus gabunensis in mice. *J Pharmacol*, 107, 2002, 145–78.

16. Osadebe P and Okeyé FBC. Anti- inflammatory effects of crude methanolic extract and fraction of Alchornea codifolia leaves. *J Ethno Pharmacol,* 89, 2003, 19–24.
17. Parimalakrishnan S, Akalankadey, Anton smith A and Manavalan R. Evaluation of anti-inflammatory, anti-nociceptive and anti-pyretic effects of methanol extract of Cleome chelidonii. *Int J Biol Chem Sci*, 1(3), 2007, 223-228.
18. Ramesh R, Petchi, Vijaya C and Parasuraman S .Anti-arthritic activity of ethanolic extract of Tridax procumbens (Linn.) in Sprague Dawley rats. *Pharmacognosy Res*, 5 (2), 2013, 113–117.
19. Rousselet MC,Vignaud JM, Hofman P and Chatelet P**.** Inflammation and inflammatory pathologies. Flammarion, Paris, 2005, 34.
20. Schorlemmer HU, Kurrle R and Bartlett R. Disease modifying activity of malononinitrilamides, derivatives of leflunomide's active metabolite, on models of rheumatoid arthritis. *Inflamm Res,* 48, 1999, 113–8.
21. Shivani G, Manish K, Gautam, Vinod K, and Raj KG. Anti-inflammatory of two classical formulation of Laghupanchamula in rats. *J Ayurveda Integr Med,* 4(1), 2012, 23–27.
22. Steiner A, Ivanov AI, Serrats J, Hosokawa H, Phayre A and Robbins. Cellular and molecular bases of the initiation of fever. *PLoS Biol,* 4, 2006, 284–96.
23. Subash RK, Cheriyan B, Parvathavarthini S, Bhaarati G and Venugopal V. Effect of polyherbal formulation rumalaya forte on adjuvant induced arthritis in rats. *Ind Drugs*, 49, 2012, 18–24.
24. Suha A, Ahmad D, Eyad Q and Talal A. Anti-arthritic activity of the methanolic leaf extract of Urtica pilulifera on albino rats. *Am J Pharmacol Toxicol*., 6 (1), 2011, 27–32.
25. Suresha R, Sushma V, Naidu, Huralikuppi and Ashwini V. Varied anti-inflammatory activity of indomethacin in different experimental animal model. *Int J Pharm Sci,* 10(3), 2012, 3993–8.
26. Tane P, Bergquis KE, TGnel M, Tchaleu Ngadjui B, Ayafor JF and Stemer O. Cyclodione, an unsymmetrical dimeric diterpene from Cylicodiscus gabunensis. Elsevier Sci Ltd., 51(42), 1995, 11595–600.
27. Tene M, Chabert P, Note O, Julbelin T, Kenla N and Tane P. Triterpenoid saponins from Cylicodiscus gabunensis. *Phyto Let*, 4, 2010, 89–92.