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# Protective Effect of Dichloromethan Extract of *Cassia alata* (Linn.) Leaves on Complete Freund's Adjuvant-Induced Inflammation in Rats.

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**ABSTRACT**

To evaluate the anti-inflammatory and protective effect of *Cassia alata* (Linn.) leaves is the objective of this study. The anti-inflammatory and protective effect of dichloromethan extract of *Cassia alata* (Linn.) leaves (CF-AECal) has been studied according to Freund's adjuvant-induced arthritic rat models. CF-AECal 50 mg/Kg, 100 mg/Kg and dexamethasone 1 mg/Kg were the doses of drugs receipt by rats. CF-AECal 100 mg/Kg showed a substantial anti-inflammatory and protective effect. CF-AECal 50 and 100 mg/Kg produced antioxidant effects in Freund's adjuvant-induced paw edema. Indeed, antioxidant enzymes are maintained more or less that their normal rate. These antioxidant effects protect the liver, the kidney and the spleen highly ($p< 0.001$). CF-AECal 50 and 100 mg/Kg lead to a significant decline in the rate of serum enzymes (AST, PAL and CREA) and a decrease of the serum concentration of total bilirubin and protein compared to animals of the control group ignited. At the doses of the extract used, the leukocyte infiltration is reduced in the cutaneous cloths of the paws of rats. In conclusion*Cassia alata* leaves extractshows anti-inflammatory and protective effects

**Keywords:** *Cassia alata*, inflammation, oxidative stress, lipid peroxidation, enzyme.

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## INTRODUCTION

Inflammation is a local reaction of the living cloths of mammals consecutive to various aggressions capable to be physical, chemical, biologic (immune answer) or infectious. Rheumatoid arthritis (RA) is a chronic auto-immune illness that reaches 1% of the world population. It is caused by an exacerbated inflammatory reaction [25]. Complete Freund's adjuvant arthritis is an established monoarthritic model of arthritis, producing the hallmark features of oedema, cartilage erosion and pain which are similar to those displayed in rheumatoid arthritis in humans [13]. As such, CFA arthritis is often used to screen potential anti-arthritic agents [4].

Extracts from the leaves of *Cassia alata* have shown several pharmacological properties such as antimicrobial and antifungal activities [33, 6], antiseptic [9], anti-inflammatory and analgesic[27] and anti-hyperglycemic [28] properties. Our previous studies showed broncho relaxant, genotoxic, and antigenotoxic effects of *Cassia alata*[26]. In Burkina Faso, some ethnobotanical studies carried out in 1996 by Professor Nacoulma/Ouedraogo on " medicinal plants and traditional medicine practices in Burkina Faso: cases of the Plateau Central Region" showed that the leaves of *Cassia alata* contain an anti-inflammatory effect. In order to consolidate the findings of this research, we have then tested these effects on animal models. The study of anti-inflammatory effects of *Cassia alata* on wistar rats is a first experiment in Burkina Faso. The fact that this test on wistar rats is the first test in Burkina Faso makes this study original. *Cassia alata* is an exotic plant that has a great geographical distribution; however it undergoes the effects of abiotic factors which characterize its habitat. Burkina Faso's climate and soil are different from Jamaica's. However the variations in temperature and rainfall influence the quality and quantity of the chemical composition of plants including *Cassia alata*. In addition within the same country, the distribution of plants varies according to the climate gradient. Thus, the photochemical profile of *Cassia alata* extract collected for instance in the northern and the central part of Burkina Faso will be significantly different. In our study, we had recommended the count back method. Thus, we split our extract with solvents of increasing polarity including hexane and dichloromethane. This makes it possible to obtain extracts which show a variable chemical composition, since these solvents do not dissolve the same secondary metabolites from plants. Since the purpose of these pharmacological tests is to work out a traditionally improved drug (MTA); we have chosen the most active extract (Extract from *Cassia alata* with dichloromethane). Lewi and his collaborators (2011) rather worked with extract from *Cassia alata* using hexane. By comparing these two works, we notice an absence of histopathologic cuts of legs of rats in Lewi's results. Histology confirmed our assumptions on the anti-inflammatory effects of *Cassia alata*. This consolidated us in our conclusions.

## MATERIALS AND METHODS

### Preparation of the plant

The leaves of *Cassia alata* were harvested in Ouagadougou at September 2013 and identified by Dr Ouedraogo of University Ouaga1 Pr Joseph KI-ZERBO, where a voucher specimen n°15965 was deposited. The leaves were dried at room temperature and crushed into powder. One kilogram of powder of *Cassia alata* was macerated at room temperature in alcohol 80% for 48 hours. Preparation was filtered using a whatman paper, concentrated in a rotary evaporator under reduced pressure and lyophilized. The yield was 13.75 %. This extract (10g) was dissolved in distilled water (75 ml). The aqueous extract obtained was introduced into a separating funnel and 75 ml of hexane was added. The mixture was shaken vigorously. The agitation was marked by periods of lower pressure in the bulb. After thirty minutes of decantation, hexane fraction which was distinguished clearly from aqueous fraction was collected. The same procedure was repeated three times with the aqueous fraction that remained. All fractions in the hexane were concentrated in the rotavapor. Dichloromethan (75 ml) was added to the aqueous fraction. The same procedure described above was followed to obtain the final fraction of dichloromethan (CF-AECal).

### Animals

*Albino swiss* rats of Wistar stump from the pet center of the Faculty Science, University Yaoundé I was used. The rats were from 7 to 9 weeks-olds and weighing 120 g to 150 g. The animals received water and food *ad libitum,* at room temperature conditions (25 ± 2°C) and 12:12 h light/dark cycle. The rats were divided into groups of five rats each. Twelve hours before the experiment animals were fasted. The rats were

anaesthetized with intraperitoneal injection of diethyl ether. The different manipulations and treatments of the animals respected the guide of the commission of animal ethics of the UO. The number of use of the animals was CEUOI 2017-05.

**Adjuvant induced arthritis in rats**

This induction founded on the method described by [24, 39]. 0.1 ml of the adjuvant has been injected in the paw of rat. Nine days after induction of inflammation, rats were treated daily with distilled water, CF-AECal (50 mg/Kg and 100 mg/Kg) or dexamethasone (1 mg/Kg) during twelve days. Normal group received only distilled water. Paw volume of rats was measured before arthritis induction, nine days after induction and every four days with a plethysmometer UGO BASILE till when 21 days.

**Study of lipid peroxidation and antioxidant enzyme activities**

Immediately after sacrifice, the liver, kidney and spleen were removed from each experimental rat and washed in the saline solution (0.9% NaCl). 10% of organs homogenates have been prepared inTris-HCL buffer (0.1M, pH 7.4). The homogenate was centrifuged at 3000 rpm x 25 min at 4°C. Supernatant was collected into sterilized tubes and stored at -20°C until analysis of reduced glutathione [8], catalase[31], superoxide dismutase activity[22] and lipid peroxidation (MDA) [37].

**Assessment of the biochemical parameters**

Blood from each rat was taken from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Clotted blood was centrifuged for 15 min at 3000 rpm and separated sera were stored at - 20° C until analysed. The separated serum was used for the estimation of some biochemical parameters such as ALAT/SGPT (Cypress diagnostics kits code HBE07), ASAT/SGOT (Cypress diagnostics kits code HBE06), Bilirubin (total and direct) (Fortress diagnostics kits code BXC0193) and ALP (Fortress diagnostics kits code BXC0184). Concentration of the biochemical constituents was calculated according to the manufacture instruction.

**Histological studies**

Paws samples have been fixed in formalin. Then tissues have been dehydrated in alcohol baths (50-100%). These tissues were cleared in xylene and included in the paraffin. Tissues have been cut at 5 µm thickness by sledge microtome Reichert-jung 2030. The different cuts have been colored to hematoxylin and eosin. These tissues are finally observed to the photo-microscopic Olympus CH-2 and are shot while adapting one camera Olympus 101 on the microscope. The observation allows to precise the degree of necrosis, fatty changes, ballooning degeneration and lymphocyte infiltration of the liver [3, 2].

**Statistical analysis**

To assess the normality and homogeneity of different distributions, the tests of Kolmogorov Smirnov and Bartlett were carried out respectively. When distributions were normal and homogeneous the analysis of variance (ANOVA) was conducted to compare several samples, followed by the post-test of Newman-Keuls. The differences were meaningful when *P< 0.05, **P< 0.0l, ***P< 0.00l.

## RESULTS

**Adjuvant induced arthritis in rats**

At the end of the experiment (on day 21), CF-AECal at the dose of 50 mg/Kg and 100 mg/Kg, inhibits respectively the paw edema at 22.81% and 36.88% compared to control animals (P< 0.001). Dexametasone 1 mg/Kg inhibits paw edema at 61.79% (Fig. 1).

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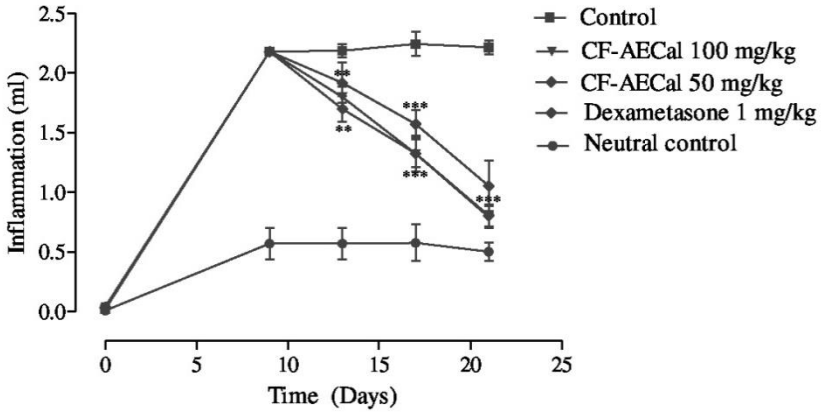


**Figure 1:** Effect of CF-AECal on CFA induced paw oedema in rats. Data are expressed as mean ± S.E.M, n = 5 *p <0.05; **p <0.01; ***p <0.001 compared to control animals. CFA = Adjuvant induced arthritis in rats; CF-AECal = dichloromethan fraction of Cassia alata aqueous extract.

**Antioxidant enzyme activities**

Three weeks after CFA administration to rats, there was a significant ($p<0.001$) increase of MDA level and SOD activity, accompanied with a significant decrease of GSH level and catalase activity in the liver, kidney and spleen. Treatment of animals with CF-AECal significantly reduced SOD activity and MDA concentration in the organs. The activity of catalase and the level of GSH were significantly increased in liver spleen and kidney of rats treated with the plant extract (Fig. 2).

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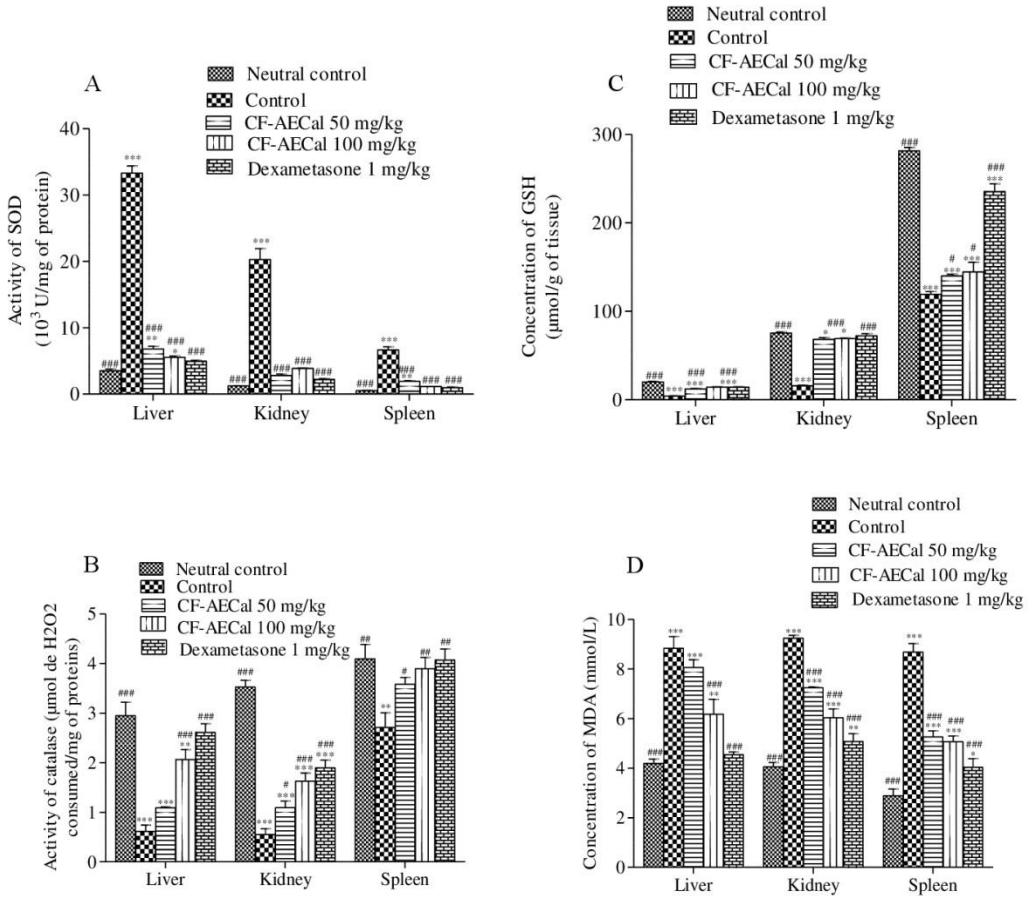


**Figure 2:** Effect of CF-AECal on CFA induced oxidative enzymes in rats. A: superoxyde dismutase activity, B: activity of catalase, C: concentration of glutathione, D: concentration malondialdehyde. The values are expressed as means± SEM, n=5 *p<0.05, ** p<0.01, ***p <0.001 compared to control animals. # p< 0.05, ## p< 0.01, ### p< 0.001 compared to neutral control animals. CFA = Adjuvant induced arthritis in rats; CF-AECal = dichloromethan fraction of Cassia alata aqueous extract.

**Assessment of the biochemical parameters**

Administration of Complete Freund's Adjuvant, caused a significant increase of serum marker enzyme in particular the transaminase (GPT/ALT and GOT/AST) and alkaline phosphatase (ALP). Similarly a significant increase of the total serumbilirubin (TB) and proteins was observed, mainly with rats from the negative control group in comparison with the animals of the neutral control group (Fig. 3). The administration of CF-AECal resulted in a significant decline of the serum (AST) and liver enzyme (ALP) activity and a decrease of the serum concentration of creatinin, total bilirubin and protein compared to CFA treated animals.

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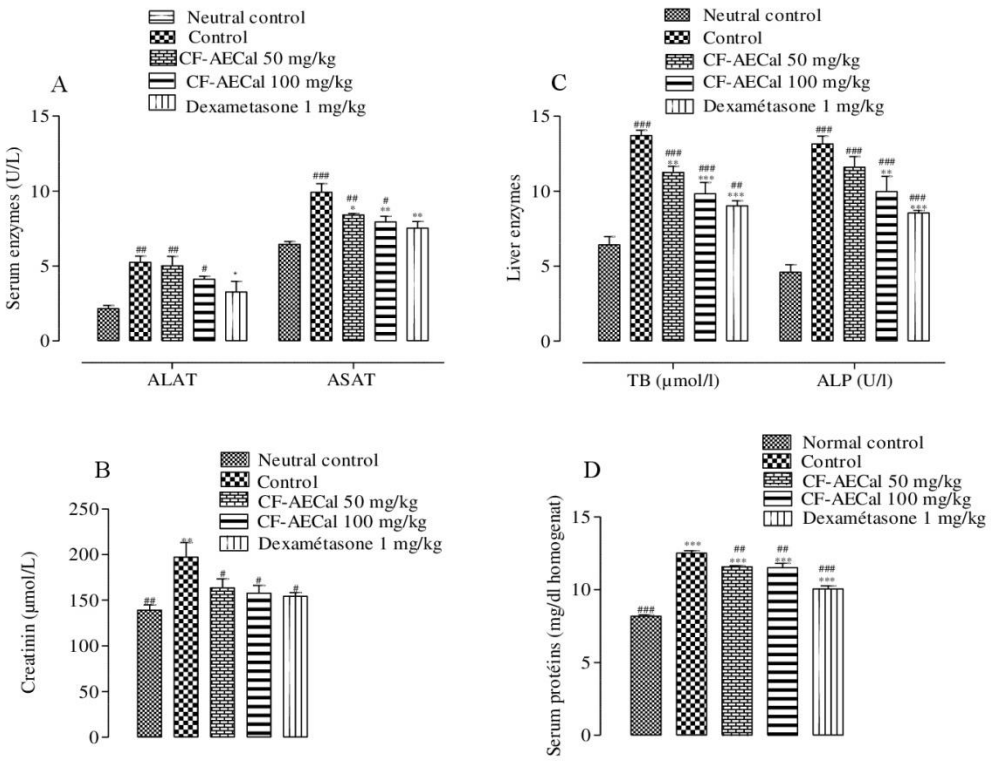


**Figure 3:** Effect of CFA and CF-AECal on some biochemical parameters. A: activity of ALT and AST (U/L), B: rate of creatinin (µmol/L), C: concentration of total bilirubin (µmol/L) and activity of alkaline phosphatase (U/L), D: concentration of proteins (mg/dl). The values are expressed as means ± SEM, n = 5.*p<0.05, **p<0.01, ***p< 0.001 compared to control animals. # p<0.05, ## p<0.01, ### p<0.001 compared to neutral control animals.

**Histological studies**

The histological analysis demonstrates that the inflammation of the paws of rats led by the adjuvant shows an infiltration of the inflammatory cells in tissues cutaneous coins. The reduction of the volume of the paw among the rats treated to the CF-AECal of 50 and 100 mg/Kg is followed of the reduction of the infiltration of the inflammatory cells in tissues cutaneous coins.

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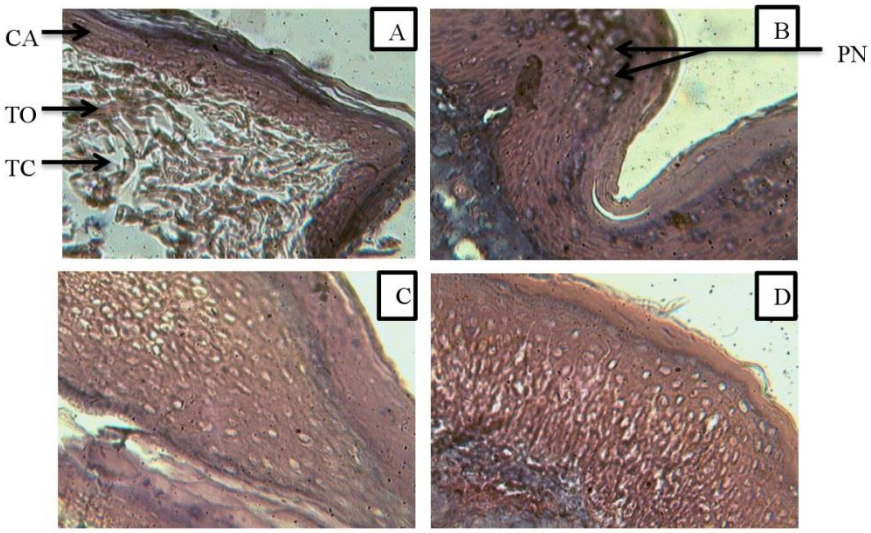


**Figure 4**: Histology of paw sections from animals of different groups (H&E x 400). A: normal rat, B: negative witness rat, C: rat treated in CF-AECal 50 mg/kg, D: rat treated in CF-AECal 100 mg/kg. CA: articular cartilage; TO: bony tissue; TC: conjunctive tissue; PN: polymorphonuclear cell.

## DISCUSSION

Adjuvant induced arthritis in rats results suggest that CF-AECal can have a potential to improve the chronic inflammation as well as acute inflammation responses. Over the past decade, it has increasingly been recognized that inflammation can induce bone damage and that the two processes are linked via common mediators. These mediators include receptor activator of NF-κB, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6, IL-17, and IL-18, and matrix-degrading enzymes [30]. The results of the biochemical analysis suggested that CF-AECal reduced the rate of these enzymes. Indeed, the destruction of the cellular membranes provokes ALT, AST, ALP increase in blood [40]. A decrease in total protein of the liver is a reflection of hepatic toxicity [10, 1]. The significant reductions of protein in the control group rats indicate depletion in the protein reserve and thus suggest hepatic toxicity.

Aerobic organisms are protected against free radicals by antioxidant defense systems. SOD, CAT and GSH are known to quench the superoxide radical and thus to prevent the damage of cells caused by free radicals [18]. Anti-inflammatory effects of CF-AECal would explain by an increase intracellular of the activity of these enzymes. We think that CF-AECal could have some molecules anti oxidizing that protect the cells.

Topical application of flavonoids (quercetin, myricetin, apigenin, chrysin) exerted a strong inhibition on cyclooxygenase (COX) and lipoxygenase[14, 35]. In addition, [38] have identified the luteolin, presents in *Cassia alata*leaves, as the more powerful flavonoid tested in the inhibition of the TNF-α, activation of the NF-κB (nuclear factor- kappa Beta) induced by lipopolysaccharide (LPS), and the activation of the AP-1 (activator protein-1). It was also demonstrated that genistein inhibits the activation of STAT-1, NF-κB, the expression of iNOS as well as the production of NO [11]. The flavonoids and the gallic acid inhibit leukocyte migration by blocking their accession to the vessel wall[20, 21] and inhibition of adhesion molecules VCAM-1, ICAM-1 and E-selectin in the vascular endothelial [34]. The tannins also have properties close to those of flavonoids: increase in the capillary strength, decrease of the capillary permeability and stabilization of collagen [5]. These tannins are ability to inhibit the phospholipase $A_2$is already established, which will participate in the inhibition of prostaglandins and leukotrienes [7]. According to [15], the gallic acid and its derivatives are responsible for the inhibition of the activation of the p38 MAPK (mitogen-activated protein kinases), and the inhibition of the fixing of the NF- κB, essential factors for the expression of the pro-inflammatory cytokines such as histamine, TNF-α and IL6. Several authors among whom[16] have demonstrated the presence in the leaves of *Cassia alata*

the components such as alkaloid, tannin, saponin, phlobatannin, terpenoid, flavonoid and cardiac glycoside. The works of [12] showed that the leaves of Cassia contain several compounds as emodin, kaempferol, phenolic acid. Polyphenols (coumarins, xanthones and tannins) effectively prevent lipid peroxidation [19, 32, 29]. The studies have shown that flavonoids are thermodynamically capable of reducing free radicals oxidants such as the superoxide, the peroxyle, the alkoxyle and the hydroxyl bytransfer of hydrogen [36] or by chelation of metal ions involved in the production of species-oxygenated reactive.

## CONCLUSION

Our results showed that CF-AECal had anti-inflammatory effects in rats. Furthermore, CF-AECal decreased the lipid peroxidation in rodents and augmented their antioxidant capacity. On the basis of the results obtained, CF-AECal effects on Freund's adjuvant-induced paw edema, at least in part may be related to an inhibition of the formation of several inflammation mediators including histamine, and its antiperoxidative and antioxidant properties.

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