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ANTI-INFLAMMATORY EFFECT OF *D. glomerata* (Frossk.) Hutch (Mimosaceae) METHANOLIC EXTRACTS IN CFA-INDUCED RAT EDEMA

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ABSTRACT

In this study, we prepared methanolic extracts of *D. glomerata*, and tested the anti-inflammatory activity of the extracts by using a rat model of adjuvant-induced edema. The extracts derived from the fruit of *D. glomerata* decreased hind paw edema after 1 day of *orally a*dministration. These results suggest methanolic extracts has anti-inflammatory.

Key words: Edema, *D. glomerata*, methanolic extract.

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INTRODUCTION

Inflammation is natural immune reaction of the body that results from an infection, injury, or illness. Inflammation has been shown to be one of the causes of arthritis (Silbernagl S and Lang F, 2000). Nitric oxide has many functions in the body as a vasodilator, diuretic and neurotransmitter (Nussler AK, Billiar TR, 1993), and is an important biomarker of the inflammatory response (Hussain *et al*., 2008). The medicinal and nutritional uses fruits and plant to cure arthritis (Martin *et al*., 1993). *D. glomerata* extract is tested it anti-inflammation activity. Various doses of *D. glomerata* extracts were applied to the animal inflammation model-paw edema experiment.

MATERIALS AND METHODS

Materials

The fresh fruits of *Dichrostachys glomerata* were collected and identified by a Botanist of the Faculty of Sciences of the University of Ngaoundéré. Once at the laboratory, fruits were cleaned and dried in an oven. The powder was mixed with the methanol and the infusion lasted 72 hours. The solvent was evaporated with the rata vapor.

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Anti-inflammatory rat experiment

Complete Freund's adjuvant (CFA, Sigma Co., USA) was used to induce rat paw edema at 1st day (pre-treatment) except control group in a chronic arthritis experimental model, and the antiedema effect of individual solvent extracts (post-treatment) was compared.

Rats were divided into 4 groups (n = 6 per group): control group, CFA (0.1 mL) only treated group (negative control), dexamethasone (1 mg/kg at 1st day, 1 mg/kg at 1h) as a positive control, sample groups *D. glomerata* extracts (25 and 50 mg/kg)] daily treated orally, over 24 days. Paw size was measured 1, 2, 4 hr and thereafter every day for 10 - 24 days.

Nitrite assay

The production of NO was measured as the nitrites that accumulated in the culture medium after colorimetric reaction with Griess reagent according to the manufacturer's manual (Cayman Chemicals, Ann Arbor,

MI, USA). In brief, samples (200 mg/ml, 20 µl, and dilution factor 10) were collected 24 hr after treatment with cultured bovine vascular endothelial (CPAE) cells. The absorbance after 5 min at 570 nm was measured with a spectrophotometer.

Malondialdehyde (MDA) assay

The assay is for Ohkawa and al., 1979. The aldehyde has action with thiobarbituric acid for give a red color. The absorbance at 500 nm was measured with a spectrophotometer (Ohkawa *et al*., 1979).

Protein assay

Proteins were measured by Biuret methods describe by Gornall et al (1949).

Catalase assay

The test use according to Sinha in 1972. The hydrogen peroxide is cut in presence of catalase. The absorbance at 570 nm was measured with a spectrophotometer (Sinha KA, 1972).

Ulcerogenic effect of the compounds on mice gastric mucosa after acute administration

Rats were put each in a separate cage deprived from food. Only water was provided for 6 h. After which, the animals received the drugs in a dose of 25 and 50 mg/kg. The animals were sacrificed after 1 h. The stomach was examined for the presence of ulcers (Whiteley PE and Dalrymple SA, 1998). An ulcer score was calculated (Martin MJ *et al*., 1993).

Statistical analysis

Mean and standard errors of all parameters were determined for each of the 6 rats. The Dunnett's -test was used to establish the significances of differences between the control and treatment groups. $p< 0.05$ was considered statistically significant.

RESULTS

Anti-inflammation

We showed that *D. glomerata*methanolic extracts have potential efficacies in treating inflammation in rats, as they significantly reduced paw edema levels and repaired damaged dorsal root ganglias of CFA adjuvant arthritis. *D. glomerata* was treated to the orally (25 and 50 mg/kg) of paw edema induced rats and the effect of extracts was in the following order: (Table. 1). The mean changes in paw edema size (mm) from 1 hr to 24 days for each group were as follows: control (0.27 ± 0.21), CFA (0.24 ± 0.00), *D. glomerata* (0.21 ± 0.00), *D. glomerata* (0.28 ± 0.00).

Pro-inflammatory cytokines (secretory NO)

Furthermore, these extracts also had multiple actions such as remarkable NO induce excretion of water and sodium (Fig. 1).

Table 1. Ulcer scores

DESCRIPTION	SCORES
No ulcer	0,00
Dilatation of vessels and small ulcer	1,00
Ulcer ≤ 4 mm	2,50
Ulcer ≥ 5 mm	5,00

Table 2. Effect of methanolic extract of *D. glomerata*on ACF inflammation

Treatment	Dose (mg/kg)	Inflammation (mL)										
		Time (hour and day)										
		Vo	V1h	V2h	V4h	24h	4 J	8 J	12 J	16J	20J	24J
Control	0	0,22	0,27	0,34	0,43	0,55	0,57	0,72	0,86	0,99	1,04	0,75
Dexamethason	1	0,22	(70,00) 0,24± 0,0*	(78,84) 0,25±0,00**	(84,57) 0,26±0,00**	(88,38) 0,26±0,02**	(89,76) 0,26±0,01**	(11,38) 0,67± 0,0 3	(36,46) 0,63± 0,03	(31,17) 0,75± 0,04	(42,35) 0,70±0,01**	(46,52) 0,51±0,01**
D. glomerata	25	0,21	(12,00) 0,26± 0,00	(9,52) 0,33±0,00	(28,70) 0,37±0,00	(33,94) 0,43±0,02	(3,91) 0,56±0,03	(7,94) 0,68± 0,04	(27,81) 67±0,02	(30,39) 0,75± 0,08	(43,64) 0,68±0,04**	(23,88) 0,62±0,03*

D. glomerata	50	0, 24	(20,00) 0,28± 0,00	(23,81) 0,34±0, 00	(24,07) 0,34±0, 00	(15,15) 0,40±0, 02	(8,38) 0,52±0, 00	(28,57) 0,57± 0,01	(43,44) 0,60± 0,01	(44,94) 0,60± 0,03	(50,65) 0,65±0, 04**	(41,42) 0,55±0, 02**

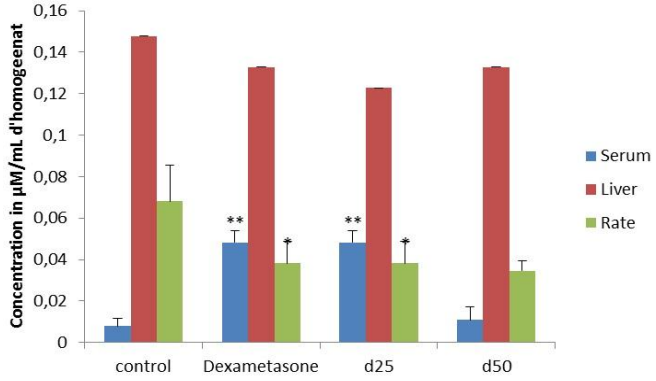
Values expressed as mean ± SEM. n= 5 in each group. Data analysis was performed using One way ANOVA followed by Dunnett's post hoc test. *P<0.05. **P< 0.01. *** compared with control. The percentage inhibitions are in bracket.

Table 2. Ulcerogenic effect of methanolic extract of *D.glomerata*

Treatment	Dose (mg/kg)	Weight of mucus (mg)	Surface of ulcer (mm^2)	Index ulcer	%Surface ulcer	Animals with ulcer %
control	0	61,54±0,20	0,00±0,00	0,00±0,00	0,00	0,00
Indomethacin	10	40,80±0,20*	7,60±0,00	2,8±0,00	76,00	100,00
D.glomerata	25	50,40±0,30*	0,00±0,00	0,0±0,10	0,00	0,00
D.glomerata	50	47,10±0,10*	0,00±0,00	0,8±0,00@	0,00	0,00

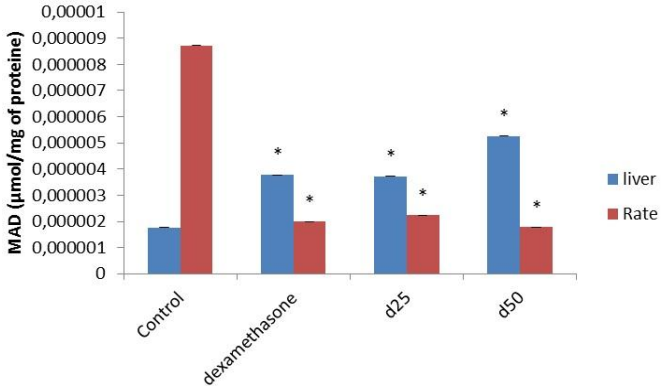
Values are expressed as mean ± SEM.*Denotes significant difference from control (p <0.05)

Fig 1. Effect of methanolic extract of D. glomerata in secretion of No on the chronic inflammation induced by Freund Complete Adjuvant (Injected paw).



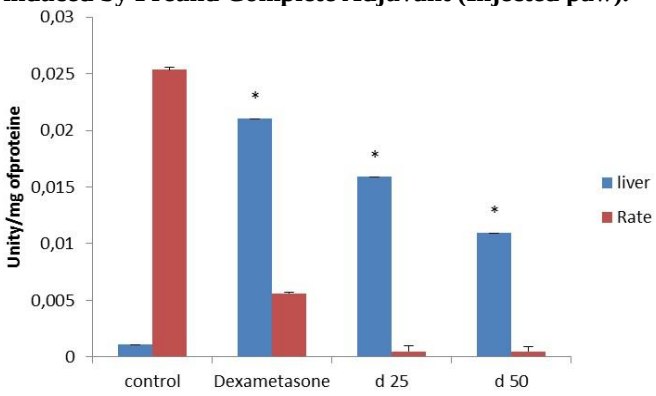
Data are given as means ± S.E.M. **p<0.01 is considered significant, compared to control

Fig 2. Effect of methanolic extract of *D. glomerata* in secretion of MDA on the chronic inflammation induced by Freund Complete Adjuvant (Injected paw).



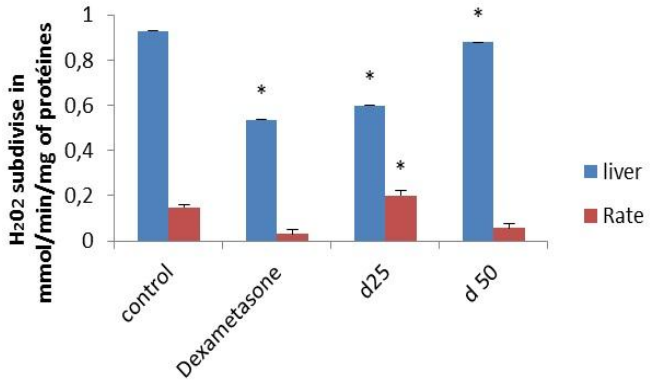
Data are given as means ± S.E.M. *p<0.05 is considered significant, compared to control.

Fig 3. Effect of methanolic extract of *D. glomerata* in secretion of protein. on the chronic inflammation induced by Freund Complete Adjuvant (Injected paw).



Data are given as means ± S.E.M. *p<0.05 is considered significant, compared to control.

Fig 4. Effect of methanolic extract of *D. glomerata* in secretion of catalase on the chronic inflammation induced by Freund Complete Adjuvant (Injected paw).



Data are given as means ± S.E.M. *p<0.05 is considered significant, compared to control.

DISCUSSION

Adjuvant induced arthritis in rats results suggest that *D. glomerata* 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 [9]. The anti-inflammatory activity of methanolic extract of *D. glomerata* was examined by using adjuvant- induced edema and arthritis in rats. Each methanolic extract, decreased the hind paw edema after 2 hour of, the extracts contributed to kidney hémodynamy on NO and reduce edema in inflammation. These results suggest that *D. glomerata* has potential as a crude ant-inflammatory drug and diuretic agent. *D. glomerata* reduce the secretion of cytokines and oxygen reactive espece, and had anti-inflammatory activity and articular cartilage destruction repair, this is deemed to be the most effective agent for this purpose.

CONCLUSION

The present study demonstrated that the methanolic extract of *D. glomerate* possess antiarthritic And ulcerogenic activity respectively. These findings may scientifically justify the use of *Dichrostachys glomerate* for the treatment of arthritis and shows that the plant can be source of new drug. However, more clinical investigations are required to substantiate this report.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954). All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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Nil

CONFLICT OF INTEREST

No interest

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