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# Analgesic, pro and anti-inflammatory activities of *Desplatsia dewevrei*; Cytokine gene expression using Wistar rats and mice

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## ABSTRACT

**Objective:** Painkilling potential of *Desplatsia dewevrei* methanol leaf extract was examined using the acetic acid-induced writhing and hot-plate tests. **Materials & Methods:** Acute anti-inflammatory effect was studied using xylene-induced ear edema and carrageenan induced paw edema models. Gene expression using RT-PCR method was used to query TNF-α, resistin and adiponectin in Wistar rats after a 3-day administration of *Desplatsia dewevrei*. **Results:** *Desplatsia dewevrei* extract significantly ($p<0.05$) decreased the number of writhes in mice at 30 mg/kg when compared to the control and Aspirin. In the hot plate induced pain test, 10 mg/kg of extract triggered comparable analgesic effect as morphine up to 2 hrs after drug administration. There was significant decrease ($p<0.05$) in xylene-induced ear oedema at 10 and 30 mg/kg doses of the extract with 80% and 30% when compared to control and dexamethasone. For Carrageenan-induced paw oedema in rats, 30 mg/kg elicited equal effect as indomethacin at 10 mg/kg respectively. The downward regulation/expression of TNF-α, resistin and adiponectin in contrast with control and the expression of beta-actin further indicates that *Desplatsia dewevrei* has both pro and anti-inflammatory activities. **Conclusion:** *Desplatsia dewevrei* methanol leaf extract is anti-inflammatory and elicits both peripherally and centrally analgesic effect.

**Keywords:** Analgesic, Inflammation, Gene Expression, *Desplatsia dewevrei*.

## INTRODUCTION

Medicinal plants continue to play a substantial role in providing valuable pharmaceutical products in health care system, agricultural, food and even cosmetic industries [1]. Many plants used as (traditional) remedies are now being endorsed through scientific research by isolation of bioactive compounds for direct use in treatments [2, 3]. The history of inflammation is as old as man on earth. It is one of the central responses of the cells and tissue to damage [4]. Inflammation is a complex physio-pathological conditions intermediated by many signalling molecules produced by leukocytes, macrophages and mast cells and the activation of complement factors that bring about oedema formation because of extravasation of fluid and proteins and the accumulation of leukocytes at the inflammatory site [5]. Several herbal medications obtained from various plant extracts have been used in the management of a wide variety of clinical maladies [6]. Though relatively little is understood about their mechanism of action, several herbal preparations have been recommended widely for inflammatory conditions [7]. According to Spoto *et al.* [8], cytokine inflammatory markers may be useful clinically in the discovery of new pro and anti-inflammatory drugs. Idyllically, a marker ought to be detectable in blood, urine samples or tissue and is either associated with a disease or is as a result of a disease. A risk marker ought to offer diagnostic, prognostic and therapeutic information.

*Desplatsia dewevrei* is a forest tree that grows up to 25 m high and 1 m girth with a widely spreading crown. It is characterised by leaves about 12-31 cm long and 4-12 cm broad, cordate on each side at base, with large white or yellow flowers [9]. *D. dewevrei* flowers most of the year and fruits between April to November yearly. The fruits are oblong-ellipsoid, 6-10 celled and longitudinally grooved about 10-20 cm long and 8-17 cm broad, yellow. It is commonly referred to as "Ikhiavboha" by the Binis' and "ila-erin" by the Yorubas'. This study was aimed at evaluating the modulatory activities of *D. dewevrei* methanol leaf extract on pro-inflammation, anti-inflammation and nociception (analgesic) in laboratory mice and rats.

## MATERIALS AND METHODS

### Plant Collection and Authentication

Leaves and fruits of *Desplatsia dewevrei* were harvested from a forest in Ugbogiobo village located in Ovia North Local Government Area of Edo State. Plant materials were identified and authenticated at the Herbarium unit in the Department of Plant Biology and Biotechnology, University of Benin, Benin City and assigned a voucher number UBHm0283.

### Plant Preparation and Extraction

Fresh leaves and fruits of *Desplatsia dewevrei* were rinsed severally under running water and air-dried in a shady place for 3 weeks. The fruits were diced evenly before drying. Plant materials were further dried in a hot air oven at 55°C for 1 hr to make them crisp and thereafter blended using a mechanical blender. Dried and blended plant materials were extracted using cold maceration method with water or methanol as solvents. Extracts were concentrated to dryness using a rotary evaporator and freeze-drier (4°C) at the Laboratory of Ecotoxicology and Environmental Forensics, University of Benin, Edo State, Nigeria.

### Pro-inflammatory and Anti-inflammatory studies

A pro-inflammatory study was carried out to investigate the expression of TNF-α, and resistin genes in laboratory rats administered with (30, 100 and 1000) mg/kg of *Desplatsia dewevrei* methanol leaf extracts for 3days following methods of RNA isolation and PCR amplification adopted by Omotuyi *et al.* [10]. Anti-inflammation was studied using carrageenan and xylene induced models in rats and mice strictly following methods described by Igbe *et al.* [11, 12-13]. Ethical consent for this study was obtained from the Department of All protocols related to animal studies were approved by the animal ethics committee of Centre for Research and Development (CRD), Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

### Primers

| Target Gene | Forward 5'-3' | Reverse 5'-3' |
|---|---|---|
| Adiponectin | TGGAATGACAGGAG CGGAAG | ACATGTAAGCGGCT TCTCCG |
| Resistin | CATACCGTTCCCAG GAAAAA | GGTGTCAGGTGGGG TAGAGA |
| TNF-α | CATCCGTTCTCTACC CAGCC | AATTCTGAGCCCGA GTTGG |
| β-actin | ACACTTTCTACAAT GAGCTGCG | ACCAGAGGCATACA GGACAAC |

### Carrageenan-induced paw oedema

Paw oedema was induced by subcutaneous injection of 0.05 ml of carrageenan (1%) into the right hind paw of Wistar rats, as was previously described [13]. Right after the carrageenan administration, the animals received an i.p. injection of saline (5 ml/kg) for animals in the control group. The animals in the test experimental groups received (10, 30 and 100) mg/kg of extract respectively. The paw volume was measured by a volume measuring instrument (Vernier calliper) at each hour point after oedema induction. Increase in the percentage of paw volume was calculated based on the volume difference between the normal and abnormal paws (with or without carrageenan injection). Four animals per group were tested.

### Xylene-induced ear oedema

Albino mice were divided into five groups of four animals each. Animals were administered orally with *Desplatsia dewevrei* extract (10, 30 and 100) mg/kg, dexamethasone (1 mg/kg) and distilled water (3 ml/kg). An hour later, oedema was induced in each mouse by applying a drop of xylene on the inner surface of the right ear using a dropper pipette. After 15 min, the animals were anaesthetized using chloroform then; 4mm of both ears were cut off using a laboratory cork borer and weighed [11]. The anti-inflammatory activity was expressed as the percentage inhibition of oedema in the treated groups compared to that of the control group.

### Analgesic studies

Hot-plate and acetic acid-induced writhing in mice were used in this study.

### Mouse writhing test

Analgesic effect of the extract was estimated by the acetic acid-induced mouse writhing test [14]. The extract (10, 30 and 100 mg/kg), acetylsalicylic acid (100 mg/kg) or 10 ml/kg distilled water were administered orally to the animals 1 hour, before intraperitoneal injection of acetic acid (0.6% v/v). The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 min for a period of 30 min.

### Ugo-basil Hot-plate test

Hot-plate was used for preferential estimation of possible centrally mediated analgesic effects of *Desplatsia dewevrei* methanol leaf extract. The central analgesic drug, Morphine was used for positive control. In this experiment, five groups (n = 4) of Swiss albino mice (20 – 25 g) were placed on Ugo basil hot-plate maintained at room temperature for 15 minutes. Group 1- negative control (3 ml normal saline p.o) and group 2- (Morphine 1mg/kg i.p.) whereas, groups 3 – 5 animals received methanol leaf extract of *D. dewevrei* (10, 30 and 100 mg/kg p.o. respectively). Each animal was independently placed gently on Ugo basil hot plate at 55°C. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined 5, 10, 15, 20, 25 and 30 minutes after administration of the drug or vehicle.

### Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analyses were carried out using one-way analysis of variance (ANOVA). Multiple comparisons were done using Tukey's multiple range tests (Graph Pad Prism 6). Significant levels were determined at $p < 0.05$. Other calculations requiring formulae (% inhibition) were determined using already established formula.

## RESULTS

### Pro Anti-inflammatory studies

*Desplatsia dewevrei* methanol leaf extract showed anti-inflammatory effects on carrageenan-induced paw oedema in using rats and xylene-

induced ear oedema in mice at 10 and 30 mg/kg in a consistent manner over 5 hours and 120 minutes after administration respectively as shown in Figures 1 and Table 1. The anti-inflammatory effect produced by *Desplatsia dewevrei* methanol leaf extract was comparable with the activities of 10 mg/kg of indomethacin and 1 mg/kg of dexamethasone which served as the reference drugs used in the individual experiments. Figure 2 shows the downward regulation of both pro-inflammatory markers; TNF-alpha; resistin and anti-inflammatory marker; adiponectin.

**Analgesic studies**

The effect of *Desplatsia dewevrei* tested on hot plate induced pain and acetic acid-induced writhing in mice showed that 30 mg/kg is the most effective dose that elicits steady analgesic effect when compared with 1 mg/kg of morphine and 100 mg/kg of aspirin which was used as positive control (standard drug). Although, there was remarkable analgesic effects were produced in animals treated with 100 mg/kg of the extract, they were not constant as time progress as shown in Figures 2 and 3 respectively.

**Table 1:** Effect of methanol extract of *Desplatsia dewevrei* on xylene-induced ear edema in mice

| Treatment | Dose (mg/kg) | Weight of Right Ear (mg) | Weight of Left Ear (mg) | Difference (mg) | Inhibition (%) |
|---|---|---|---|---|---|
| Control | 3 ml/kg | 0.0063 ± 0.0001 | 0.0074 ± 0.0001 | 0.0010 ± 0.0006 | - |
| *Desplatsia dewevrei* | 10 | 0.0063 ± 0.0002 | 0.0062 ± 0.0002 | 0.0002 ± 0.0006 | 80 |
| *Desplatsia dewevrei* | 30 | 0.0067 ± 0.0004 | 0.0060 ± 0.0004 | 0.0007 ± 0.0006 | 30 |
| *Desplatsia dewevrei* | 100 | 0.0069 ± 0.0009 | 0.0053 ± 0.0057* | 0.0016 ± 0.0006 | -60 |
| Dexamethasone | 1 | 0.0060 ± 0.002 | 0.0047 ± 0.004* | 0.0014 ± 0.0006 | -40 |

Data are the mean ± SEM values for four mice in each group. *p< 0.05 as compared to the control and standard drug

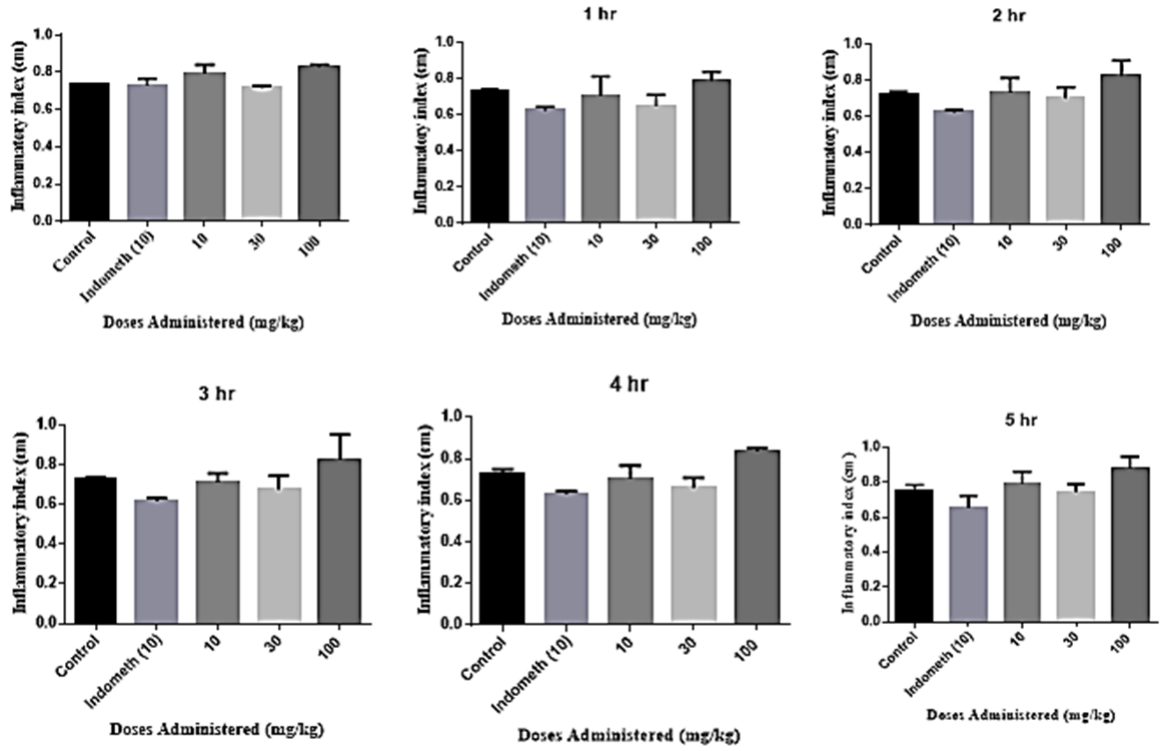


**Figure 1:** Effect of *Desplatsia dewevrei* on Carrageenan-induced Paw oedema in Rats

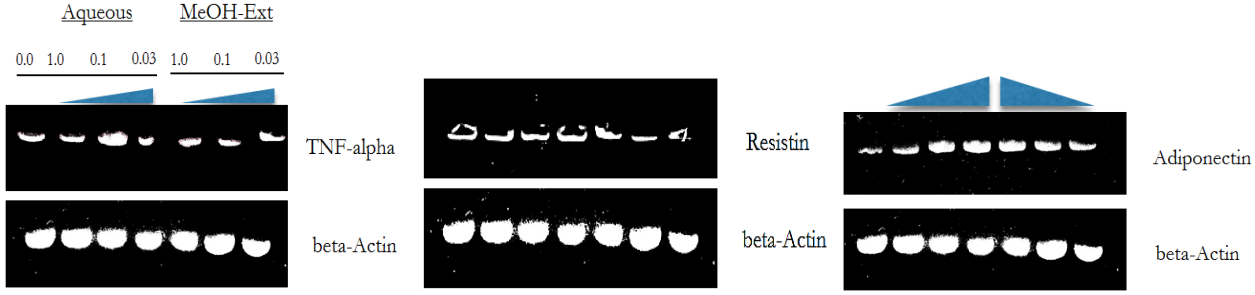


**Figure 2:** Anti-inflammatory and pro-inflammatory effects of *Desplatsia dewevrei* using anti-inflammatory (Adiponectin) and pro-inflammatory (TNF-α; resistin) markers.

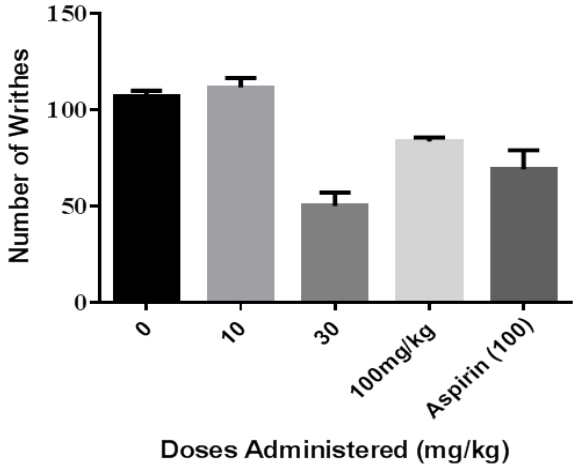


**Figure 3:** Effect of aqueous extract of *Desplatsia dewevrei* on acetic acid-induced mouse writhing.

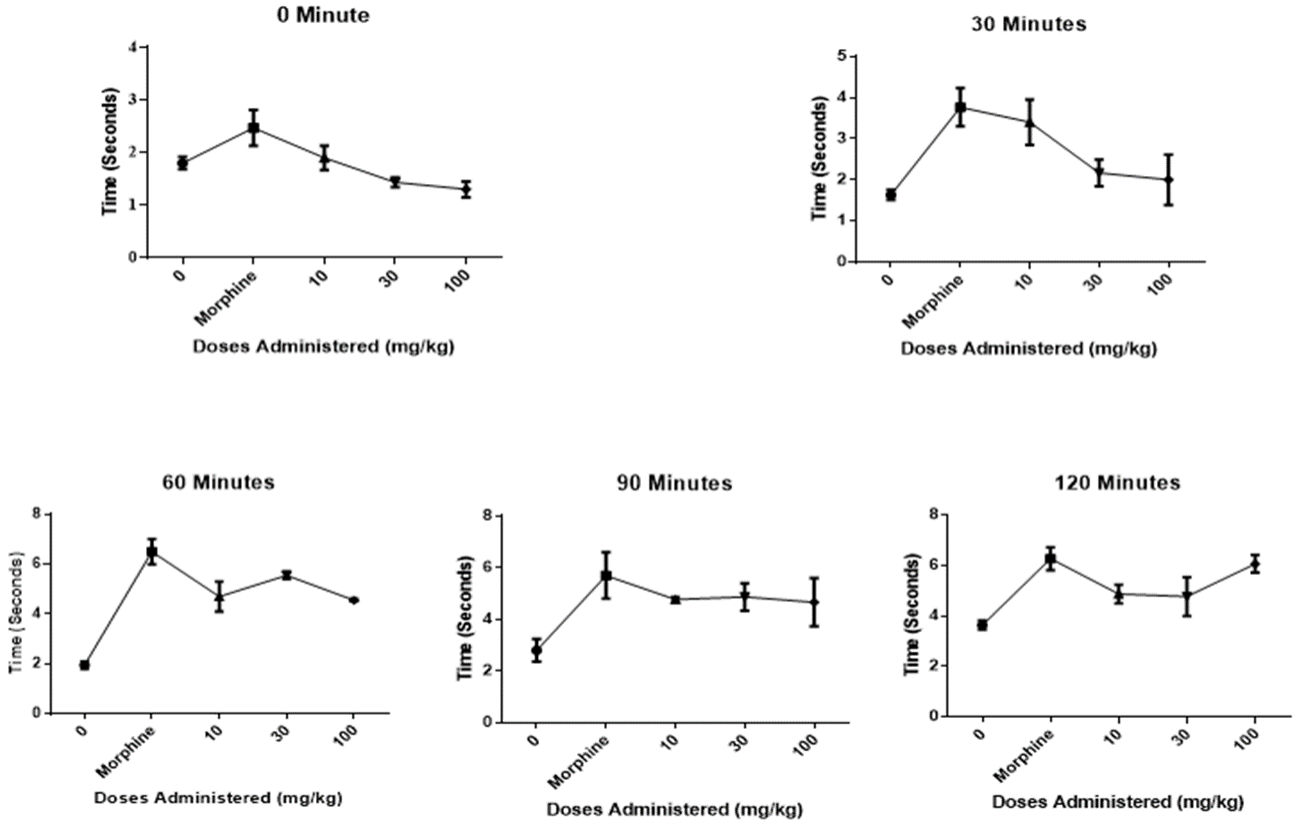


**Figure 4:** Effect of *Desplatsia dewevrei e*xtract on hot-plate-induced pain in mice

## DISCUSSION

### Anti and pro-inflammatory studies

Inflammation is involved in the physiopathology of a number of diseases comprising cardiovascular disease, cancer, diabetes, age-related macular degeneration [15-20] and possibly depression [21].

Carrageenan-induced swelling in rats is a well-known model for estimating anti-inflammatory drugs that potentiate their effects in the central nervous system. *Desplatsia dewevrei* methanol leaf extract at 30 mg/kg dose elicited competitive activities with 10 mg/kg indomethacin which served as the reference drug in the study (Figure 1). Conversely, the results of xylene-induced ear oedema in this study suggest that the effect of *D. dewevrei* extract inhibits phospholipase in a similar manner or even better manner than that provided by anti-inflammatory steroids such as dexamethasone (Table 1). Anti-inflammatory activity elicited by *D. dewevrei* is in contrast to the results of Igbe *et al.* [11] in their report on the anti-inflammatory activity of *Piliostigma thonningii* (Fabaceae) aqueous leaf extract. The remarkable anti-inflammatory activities potentiated by *D. dewevrei* methanol leaf extract for both the carrageenan and xylene-induced

inflammation models were most evident at 10 and 30 mg/kg dose (Table 1 and Figure 1).

Pro-inflammatory cytokines are produced chiefly by stimulated macrophages and are involved in the upward regulation of inflammatory responses. TNF-α which is an inhibitor of pro-inflammatory cytokines are involved in the process of neurotic pain [22, 23]. TNF-α has been shown to play important roles in inflammatory, thermal and neuropathic hyperalgesia [24, 25]. Adiponectin concentrations have been found to be inversely associated with systemic inflammation and increased concentrations of high-sensitive C-reactive proteins [26, 27]. Plasma resistin levels have been established associated with several inflammatory markers in some pathophysiological conditions [28]. An earlier reported study by Stejskal *et al.* [29] found that individuals showing clinical signs of serious inflammation showed significantly higher concentrations of resistin than healthy individuals. This implies that instances of austere inflammations have a significant positive correlation between resistin and inflammatory markers. Figure 2 in this study shows a downward regulation of TNF-alpha, resistin and adiponectin indicating that treatment of experimental animals with *Desplatsia dewevrei* leaf extract triggered no macrophage activation in the liver which is a major site of drug metabolism. This lays credence to the ability of the herbal extract to inhibit inflammation in laboratory animals exposed to carrageenan and xylene.

**Analgesic studies**

Acetic acid-induced mouse writhing experiment have been widely used to qualify analgesic agents that have peripheral analgesic activity [30]. In this study, the methanol extract of *D. dewevrei* leaves demonstrated significant analgesic activities at 10 mg/kg and 30 mg/kg doses in the hot-plate writhing analgesic models consistently after administration for two (2) hours (Figure 4). The analgesic activities of *D. dewevrei* were consistent from the time of administration to after thirty (30) minutes from the time of administration at 30 mg/kg in the acetic-acid induced writhing test. Graphically, *Desplatsia dewevri* potentiated better analgesic effect than 100 mg/kg Aspirin but statistically, the activities of the reference drug (100 mg/kg Aspirin) and the plant extract elicited activities that were statistically the same (Figure 3). From the results from this aspect of the present research, conclusions can be made that *D. dewevrei* extract has peripheral and centrally mediated mechanisms as corroborated by the study of Khatoon *et al.* [31]. According to Timothy and Idu [32], phytochemicals such as alkaloids, tannins, steroid and saponins present in medicinal plants may be responsible for their analgesic activities.

## CONCLUSION

In conclusion, the methanol leaf extract of *Desplatsia dewevrei* at 30 mg/kg and below possess affirmative analgesic effect mediated through peripheral and centrally mediated inhibiting mechanisms. The ability of the extract to down-regulate the expression of pro and anti-inflammatory markers in this study attests to the anti-inflammatory properties of *D. deweveri*; justifying its use in the treatment of pain and swellings in ethnomedicinal practices.

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