

EFFECTS OF THE ETHANOLIC EXTRACT OF *BULBOSTYLIS COLEOTRICH* (HOCHST. EXA. RICH.) ON INFLAMMATION IN ADULT WISTAR RATS

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ABSTRACT

Bulbostylis coleotricha (family Cyperaceae) is a stem-tufted plant found in tropical region of Africa. It is locally used in treating various ailments among the African folks. Despite the high esteem this plant is held in Africa, there is dearth of scientific studies on *B. coleotricha*. This, in addition to the alarming increase in inflammatory diseases around the world, makes the present study to focus on the effect of the *B. coleotricha* extract on laboratory induced inflammation. Twenty-five adult Wistar rats, weighing between 225-287g were used for this experiment, and were randomly divided into five groups of five rats per group. The groups were labelled and treated as follow: Group 1, the negative control group, was treated with 10 ml/kg body weight (*b. w.*) of distilled water. Groups 2, 3 and 4, the treated groups, were treated with 25 mg, 50 mg and 100 mg/kg *b. w.* of ethanolic extract of *B. coleotricha* respectively. Doses were based on the local information on *B. coleotricha* usages. Group 5, the positive control group, was treated with 5mg/kg *b. w.* of indomethacin. In all the groups, the effects of all treatments were checked on induced inflammation using cotton pellet granuloma and carrageenan-induced paw oedema model. The results in the two models used showed that ethanolic extract of *B. coleotricha* significantly inhibited inflammation in the treated groups. In the cotton pellet granuloma test the extract at the dose of 100 mg reduced granuloma formation from 30.2 ± 2.4 mg to 11.2 ± 2.6 mg reduced paw oedema from 7.0 ± 1.7 mm to 2.4 ± 0.8 mm (control is 100 mg/kg). In conclusion, this study established that *Bulbostylis coleotricha* plant is effective in the treatment of inflammation and can be explored in the treatment of various diseases with underlying inflammation.

INTRODUCTION

Bulbostylis coleotricha is tiny annual sedge commonly found in tropical region of Africa. The plant, which is also sometime called *Fimbristylis coleotricha*, is a stem tufted plant of about 4-10 inches long. The leaves, which are about one third to half the length of the stem, are sheaths with long white hairs in the throat. The plant has umbel which is about 5-1 of solitary spikelet. The spikelet is oblong-ellipsoid, rusty-brown with a few long white hairs. Its glumes are boat-shaped, ovate, keel yellowish and minutely excurrent. *Bulbostylis coleotricha*'s nut is hardly half of the length of the glume. It is trigonous, oblong-ellipsoid, pale yellow, obscurely striated lengthwise and horizontally barred [1]. This plant is used alone or together with other plants by local folks in treating various illnesses. The effect of the plant on inflammatory processes has not been validated, hence the focus of this study.

Inflammation is a dynamic process induced by stimuli from mechanical injuries, noxious stimuli, microbial infection and burn that may threaten the health of the host [2]. It starts by changes in blood flow, increased vascular permeability and production of whopping amount of proinflammatory cytokines such as tumour necrosis factor α , interleukin-12, reactive nitrogen and oxygen intermediates [3,4,5]. In addition to this, lipid mediators from arachidonic acid also form part of the earliest signal released in response to various inflammatory insults [6]. This arachidonic acid is released from membrane phospholipids during cell activation that begins the inflammatory process. The enzymes required in metabolizing arachidonic acid to form lipid autacoids are cyclooxygenases and the lipoxygenases [7]. These two enzymes

lead to formation of prostaglandins and thromboxanes (cyclooxygenases) as well as leukotrienes and 5S-hydroxy-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid (lipoxygenases) [2].

The fact that inflammation is now known to be the underlying basis for the pathogenesis of various renowned diseases makes the development of new anti-inflammatory drugs always wanted and welcomed. In addition to this, recent shift of interest from synthetic drugs to natural products around the world further instigated us to focus and investigate the pharmacological potential of ethanolic extract of *Bulbostylis coleotricha* plant on laboratory induced inflammation.

Anti-inflammatory evaluations of drugs or plants' extract are done using various experimental inflammatory models in the laboratory. Such models include induced arthritis model [8], Cotton pellet granuloma model [9], and carrageenan-induced paw oedema model [10] among other. In this study, cotton pellet granuloma model and carrageenan induced paw oedema model were used to evaluate the potency of ethanolic extract of *B. coleotricha*.

RESULTS

Cotton Pellet - Induced Granuloma Model

The results of the harvested granuloma tissue formed around the cotton pellet in the treated groups showed a dose-dependent and significant ($p < 0.05$) decrease in the weight of the granuloma

tissue when compared with the control (Table 2). The 100 mg/kg group had the lowest weight of granuloma (11.2 ± 2.6 mg) and the highest percentage (Fig. 1) of granuloma inhibition (60%). All the

treated Group 2, 3 and 4 decreased significantly at compared with the control. Reference group 5 also decreased significantly at $p < 0.05$ compared with the control.

Table 1: Effect of Ethanolic Extract of *B. coleotricha* on Cotton Pellet Induced Granuloma in Wistar Rats

Group	Weight of Granuloma Tissue (mg)
Group 1	30.2 ± 2.4
Group 2	$19.6 \pm 2.7^*$
Group 3	$14.8 \pm 2.1^*$
Group 4	$11.2 \pm 2.6^*$
Group 5	$16.4 \pm 2.3^*$

Each value is the mean $\pm$ SEM of 5 rats. * = $p < 0.05$ compared with control, ANOVA. Group 1- Distilled water (10 ml/kg *b.w.*); Group 2- ethanol extract of *B. coleotricha* (25 mg/kg *b.w.*); Group 3- ethanol extract of *B. coleotricha* (50 mg/kg *b.w.*); Group 4- ethanol extract of *B. coleotricha* (100 mg/kg *b.w.*); Group 5- Indomethacin (5 mg/kg *b.w.*).

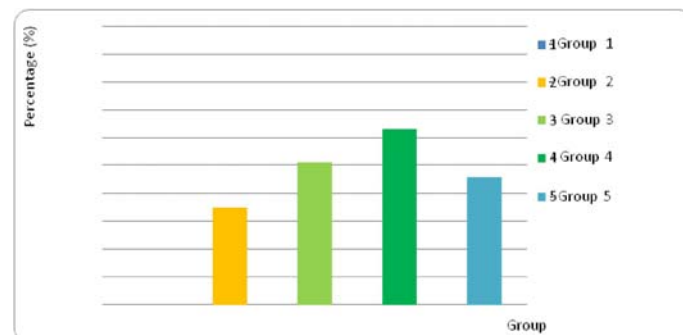


Fig. 1: Percentage Inhibition of Ethanolic Extract of *B. coleotricha* on Cotton Pellet- Induced Granuloma in Wistar Rats

Group 1- Distilled water (10 ml/kg *b.w.*); Group 2- ethanol extract of *B. coleotricha* (25 mg/kg *b.w.*); Group 3- ethanol extract of *B. coleotricha* (50 mg/kg *b.w.*); Group 4- ethanol extract of *B. coleotricha* (100 mg/kg *b.w.*); Group 5- Indomethacin (5 mg/kg *b.w.*); n- 5 rats.

Table 2. Effect of Ethanolic Extract of *B. coleotricha* on Carrageenan-Induced Inflammation in Wistar Rats.

Group	Paw size (mm) 3hrs	Paw size (mm) 5hrs
Group 1	7.0 ± 1.7	5.0 ± 1.2
Group 2	$2.8 \pm 0.4^*$	$1.0 \pm 0.3^*$
Group 3	$1.4 \pm 0.8^*$	$0.4 \pm 0.2^*$
Group 4	$2.4 \pm 0.8^*$	$0.8 \pm 0.8^*$
Group 5	$0.8 \pm 0.4^*$	$0.0 \pm 0.0^*$

Each value is the mean $\pm$ SEM of 5 rats. * = $p < 0.05$ compared with control, ANOVA. Group 1- Distilled water (10 ml/kg *b.w.*); Group 2- ethanol extract of *B. coleotricha* (25 mg/kg *b.w.*); Group 3- ethanol extract of *B. coleotricha* (50 mg/kg *b.w.*); Group 4- ethanol extract of *B. coleotricha* (100 mg/kg *b.w.*); Group 5- Indomethacin (5 mg/kg *b.w.*).

Carrageenan-Induced Inflammation Model

The results of carrageenan-induced inflammation showed significant ($p < 0.05$) decrease in paw size of all the treated groups compared with that of the control (Table 3). At 3 hours post-carrageenan administration, the paws of the extract-treated animals (Groups 2, 3 and 4) decreased significantly ($p < 0.05$) compared with the control group. The same trend of results was observed after 5 hours of carrageenan injection. The 50 mg/kg dose produced the highest reduction of paw oedema (1.4 ± 0.8 mm) compared with the other extract doses.

Percentage Inhibition of Ethanolic Extract of *Bulbostylis coleotricha* on Carrageenan-Induced Inflammation model

Percentage inhibition in all groups is quite remarkable and increased with time (Fig. 2). At 3 hours post-carrageenan injection, inhibition in the treated groups and that of the reference group was beyond 50%. The group treated with 50 mg/kg *b.w.* of the extract recorded the highest inhibition among the treated groups. Two hours later (at 5 hours), inflammatory inhibition continued to improve and increase. The least inhibition recorded was in the low dose of 25 mg/kg *b.w.* of the extract while the highest inhibition of 92% was recorded in medium dose of 50 mg/kg *b.w.*

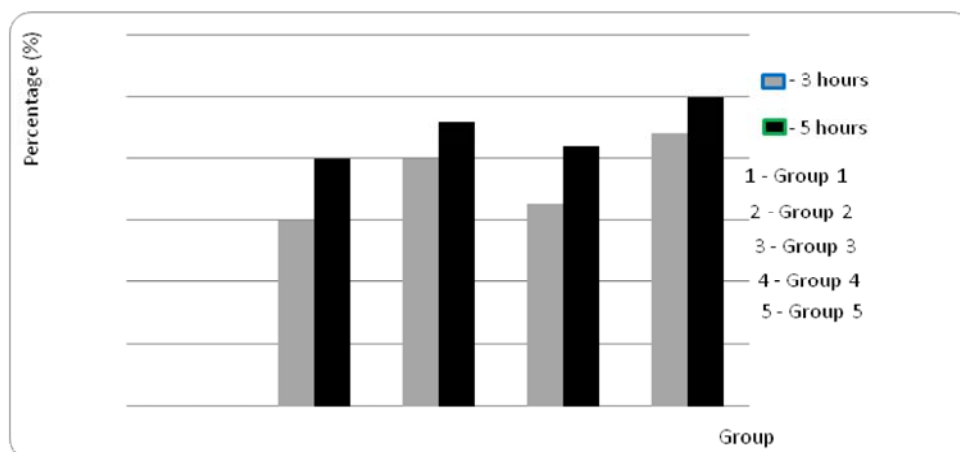


Fig. 2: Percentage Inhibition of Ethanollic Extract of *B. coleotricha* on Carrageenan-Induced Inflammation in Wistar Rats.

Group 1- Distilled water (10 ml/kg *b.w.*); Group 2- ethanol extract of *B. coleotricha* (25 mg/kg *b.w.*); Group 3- ethanol extract of *B. coleotricha* (50 mg/kg *b.w.*); Group 4- ethanol extract of *B. coleotricha* (100 mg/kg *b.w.*); Group 5- Indomethacin (5 mg/kg *b.w.*); n- 5 rats.

DISCUSSION

Despite the fact that inflammation is known to be an important part of pathogenesis of various known diseases, its treatment is still subjective to the patients or the diseases. This has resulted into different investigations on drugs that can successfully treat inflammation of all forms. This is necessary because present drugs are not giving wholly satisfactory result. The non-steroidal anti-inflammatory agents for instance have gastrointestinal side effects as a major problem [2]. Thus, safe and potent new sources of anti-inflammatory agents are always welcomed.

In the two models used, ethanollic extract of *B. coleotricha* at all doses significantly decrease and inhibit inflammation to a minimal level within the time frame of the study. In other words, ethanollic extract of *B. coleotricha* at all doses significantly treat or inhibit both acute and chronic inflammation. Carrageenan-induced paw swelling and the cotton pellet-induced granuloma in this study were selected to represent models of acute (exudative phase) and chronic (the proliferative phase) of inflammation respectively. In fact, the extract effectively inhibited granuloma formation much better compared to an established drug, indomethacin. The results, were in line with previous trend where various plant extracts in past studies were shown to have anti-inflammatory properties. For instance, ethanollic extracts of *M. piperita*, *J. officinalis*, *B. vulgaris*, and *C. molmol* possessed anti-inflammatory effect on both acute and chronic inflammation [9].

The results in this study however demonstrated strong potency of ethanollic extract of *B. coleotricha* in the treatment of inflammation. This is because at low dose of 25 mg/kg *b. w.*, the crude extract was able to inhibit inflammation in the two models used in this study. The implication is that isolation of the active constituent in this extract might lead to the use of very small dose in treating various inflammatory conditions. This might limit the chance of any possible side effect from the active constituent of

B. coleotricha (though not investigated in this study) and also reduce the cost of production since little milligram of the active constituent will be needed for treatment of inflammatory condition.

The above inhibitory effect of *B. coleotricha* in the inflammatory models used in this study could be to be due to the presence of flavonoids and phenolic compounds in the extract (Table 1). This is because phytochemical analysis of the extract showed that alkaloids and flavonoids were present in the extract. Also, phenols, saponins and quinines were also documented to be present in the ethanollic extract (Table 1). Various medicinal plants in the family of Convolvulaceae have been implicated for their anti-inflammatory action due to presence of phenolic compounds constituents [11,12]. Also, anti-inflammatory effects of *Ocimum basilicum* L. was reported to be possibly via blockade of cyclooxygenase (COX) and thus inhibition of carrageenan-induced, arachidonic acid-and leukotriene-induced paw oedema, an action that was believed to be due to the presence of phenolic compounds in the plant [2,13]. Apart from this, flavonoids had been proven to have anti-inflammatory effects. Though over 4000 different flavonoids have been documented, these polyphenolic compounds were implicated to have a variety of biological effects in numerous mammalian cell systems both *in vitro* and *in vivo* [8,14]. Formica and Regelson [15] claimed that flavonoids can exert anti-inflammatory, antioxidant, antimicrobial, antiviral, antiulcerogenic and cytotoxic effects among others. *In vivo* studies of anti-inflammatory activity of flavonoids showed inhibition of paw oedema caused by croton oil as well as inhibition of the development of induced granuloma [16,17].

CONCLUSION

In conclusion, *B. coleotricha* plant extract was effective in the treatment of both acute and chronic inflammations as demonstrated in the inflammatory models in this study. The plant can thus serve as a cheap source of anti-inflammatory agent for the treatment of various inflammatory diseases.

MATERIALS AND METHODS

Plant Material and Extraction

Bulbostylis coleotricha was collected in April around the Post-graduate Hostel of University of Ilorin, Nigeria. The plant was subsequently identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Having been identified by Messrs. Ngbogu, O. A. and Shasanya, O. S., and finally confirmed by Mr. Onadeji, M. O. A herbarium specimen was deposited and a voucher number (FHI 108793) was obtained. The preparation of plant extract was done by air-drying, pulverizing and macerating 960 g *B. coleotricha* in 5 litres of ethanol for 3 days at room temperature. The mixture was later filtered, using a funnel lined with a Whatman filter paper. The filtrate was later evaporated at a regulated temperature of 50°C in a water bath. The yield of 12.8 g of dark brown semi-solid extract was obtained from the plants. Stock solution was prepared by mixing the extract with distilled water for oral route treatment. Doses used in this experiment were 25 mg-, 50 mg- and 100 mg/kg *b. w.* of the extract; while the control was administered with 10 ml/kg *b. w.* of distilled water. The stock solution of the extract was prepared by dissolving 5000 mg of the semi-solid extract into 200 ml of distilled water. Doses were calculated in accordance with the weight of each animal, treatments were done orally in all groups.

Animals

Fifty male and female adult Wistar rats (in ratio 3:2), weighing between 225 g– 287 g, and obtained from the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, were used for this study. The rats were allowed to acclimatize for 10 days with food and water available *ad libitum*. Animals were then randomly assigned into five (5) different groups and housed in a group of 5 rats in standard see-through wooden cages at the Animal House of Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria. Groups 2, 3 and 4 were treated with the 25 mg, 50 mg and 100 mg/kg *b. w.* of the extract; while Groups 1 was given 10 ml/kg *b. w.* of distilled water and Group 5 was treated with 5 mg/kg *b. w.* of Indomethacin. All studies were performed in accordance with the University of Ilorin's Guide for the Care and Use of Laboratory Animals [18].

Drug

Indomethacin produced by Chiest Farmaceutical S.P.A. (43100), City Parma, Italy, was used at 5 mg/kg *b. w.* for the rats in Group 5. The control was however administered with 10 ml/kg *b. w.* of distilled water.

In vivo Experiment

The two models used for these studies were cotton pellet granuloma and carrageenan - induced paw oedema model. In these models, extract was tested at the doses of 25 mg-, 50 mg- and 100 mg/kg *b. w.* for possible anti-inflammatory action by comparing these groups with the control and reference group subjected to the same models but treated with distilled water (10ml/kg *b. w.*) and indomethacin (5mg/kg *b. w.*) respectively.

Cotton Pellet Granuloma Model

Anti-inflammatory activity of ethanol extract of *B. coleotricha* plant was evaluated as described by Mossa et al[23]. Briefly, a sterilized cotton pellet weighing 30mg was implanted subcutaneously in the groin of each rat. The animals were then given orally 25mg- (Group 2), 50mg- (Group 3) and 100mg/kg *b. w.* (Group 4) of ethanolic extract of *B. coleotricha* for seven (7) consecutive days, while the animals in the control (Group 1) and reference (Group 5) groups received distilled water (10ml/kg *b. w.*) and indomethacin (5mg/kg *b. w.*) respectively. On the 8th day, animals were sacrificed with an overdose of ether as anaesthetic; and the cotton pellet surrounded by granuloma tissue was dissected out carefully. This was dried to a constant weight at 60°C for 12 hours. Mean weight of the granuloma tissue formed around each pellet was obtained and percentage inhibition was calculated for each group.

Carrageenan - Induced Paw Oedema Model

Anti-inflammatory activity of ethanol extract of *Bulbostylis coleotricha* plant was also assessed simply using the method described by Winter *et al* [10]. Basically, it involves inducing inflammation by injecting 0.1ml of 1% carrageenan into the right hind paw of each rat under the plantar aponeurosis. The animals in the treated group were orally treated with 25mg-, 50mg- and 100mg/kg *b. w.* of ethanol extract of *B. coleotricha* 1hour before carrageenan injection. Though subjected to the same procedure, the animals in the control and reference groups received distilled water (10ml/kg *b. w.*) and indomethacin (5mg/kg *b. w.*) respectively. Paw size was measured, as done by Owoyele *et al* [20], by wrapping a piece of cotton thread around the paw and the circumference measured with a meter rule. This measurement was carried out immediately after injection of carrageenan; and subsequent measurement were made between 1-5hrs following carrageenan injection. Inhibitory activity at 3hrs was emphasized as a measure of extract potency and the inhibitory activity was calculated according to the formula below:

$$\% \text{ inhibition} = \frac{(C_t - C_o)_{\text{control}} - (C_t - C_o)_{\text{test}}}{(C_t - C_o)_{\text{control}}} \times 100;$$

where C_o is paw size before inhibition and C_t is paw size after inhibition.

Statistics

Statistical analyses on results were performed using SPSS 16 (SPSS Inc., Chicago, IL, USA). Results were presented as means $\pm$ standard error of the mean (SEM) for 5 rats per group using one

way analysis of variance (ANOVA). Significant differences were accepted at $p < 0.05$.

Recommendations

The authors wish to suggest that further studies be done to isolate the active components responsible for the anti-inflammatory effects such that these could be subjected to the same processes.

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