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Research

Evaluation of anti-inflammatory activity of 2,3-dimethylnaphthalene in carrageenan and cotton pellets-induced inflammation models

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Abstract

Some naphthalene derivatives possess anti-inflammatory activity. This study evaluated the effects of different doses of 2,3-dimethyl naphthalene on two inflammatory models. Thirty male albino rats were assigned into six groups of five rats each. Group I: normal, group II: untreated control, group III: treated with 10 mg/kg *b.w* diclofenac, groups IV-VI treated with 5, 10, and 15 mg/kg *b.w.* 2,3-dimethyl naphthalene, respectively. Carrageenan-induced paw edema and cotton pellets-induced granuloma were used to evaluate the anti-inflammatory activity of 2,3-dimethyl naphthalene. GraphPad Prism® was used to analyze data. Data were expressed as mean ± standard error of the mean at a significance level of $p < 0.05$. The study revealed that 5, 10, and 15 mg/kg *b.w*. 2,3-dimethyl naphthalene suppressed paw edema by 55.82%, 21.49%, and 14.38% respectively, while 10 mg/kg *b.w.* diclofenac sodium suppressed paw edema by 81.50%. Furthermore, 5, 10 and 15 mg/kg *b.w* 2,3-dimethyl naphthalene significantly *($p < 0.05$)* inhibited cotton pellets-induced granuloma by 11.48%, 38.52% and 39.69%, respectively, while 10 mg/kg *b.w.* diclofenac sodium inhibited cotton pellets-induced granuloma by 59.28%. Thus findings from this study showed that 2,3-dimethyl naphthalene could exhibit anti-inflammatory actions and its mechanism might be through interference with phase 2 of inflammatory stressors.

Keywords: 2,3-dimethyl naphthalene, carrageenan, cotton-pellets granuloma, paw edema inflammation

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Introduction

Inflammation is part of the body's defense mechanism against detrimental insults such as microbial infections, tissue injury, or damage (Lim *et al.,* 2019; Sheng *et al.,* 2019). Such insults lead to the accumulation of fluids and leukocytes in the extracellular tissues leading to destroying, diluting, or walling off the injurious agents and initiate the repair process and healing (Chen *et al.,* 2018). Inflammation can be acute, occurring within a short time, or chronic resulting in prolonged or uncontrolled inflammation (Vetriselvan *et al.,* 2013). Chronic inflammatory diseases are the most significant cause of death worldwide and the World Health Organization rated them as the greatest threat to human health (Kayode *et al.,* 2020; Pahwa *et al.,* 2019).

Conventional treatment of inflammatory diseases which includes the use of non-steroidal and steroidal anti-inflammatory drugs is associated with an increased risk of health issues such as gastrointestinal disorder, immune compromise, and humoral disturbances, when it is used for a long time (Cai *et al.,* 2014). World Health Organization also stated the intense side effects of anti-inflammatory drugs among other reports, therefore, there is a need for concerted efforts to find an alternative solution to inflammatory diseases. Researches are ongoing to discover drugs with the capacity to serve as therapeutic agents against numerous diseases. Naphthalene and its derivatives have shown anti-inflammatory, antibacterial, anticancer, antimicrobial, and immunomodulatory properties (Pandya *et al.,* 2012). 2,3-dimethyl naphthalene, a triterpenoid, and one of the suspected bioactive compounds present in hexane fraction of *Costus afer* leaves have been shown to possess anti-inflammatory properties (Anyasor *et al.,* 2014; Anyasor *et al.,* 2015). It is a colorless solid that forms a shining flaked-crystal; it has a melting point of 82.2^{o}C, and it possesses a familiar odor of mothballs. It is very volatile and sublimes slowly at room temperature. Naphthalene is insoluble in water, moderately soluble in alcohol, highly soluble in ether, and benzene (Rokade & Sayyed, 2009).

Hence, this study was designed to evaluate the effects of 2,3-dimethyl naphthalene on two *in vivo* inflammatory models.

1.0. Materials and methods

1.1. Chemicals and reagents

2,3-dimethyl naphthalene, Carrageenan, and cotton pellet were purchased from Tokyo Chemical Industry (Fischer Scientific, Massachusetts, USA). Corn oil was purchased from Super Vitec Edible Oils Sdn. Bhd. Nigeria. Diclofenac sodium was purchased from Hovid Pharmaceuticals, Nigeria. All other chemicals and reagents used were of analytical grade.

1.2. Acute toxicity study

The acute toxicity study for 2,3-dimethyl naphthalene was performed following the Up and Down Trial and Error Method as described by the Organization for Economic Cooperation and Development (OECD) guideline 425 (OECD, 2008). Before dosing, animals were fasted overnight and then weighed. The dose was calculated according to body weight, after the compound administration, food was withheld for another 3-4 hours. Animals were dosed 100 mg/kg body weight (*b.w.*) one at a time and observed for 24 hours, the dose for the next animal was increased by a factor of 3.2 to achieve a maximum dose of 320 mg/kg *b.w*. (Erhirhie *et al.*, 2018). The animals were monitored for clinical signs of toxicity for 48 hours and further monitoring was done for 14 days to check for delayed toxicity (Enegide *et al.*, 2013).

2.2.1. Wellness parameters

Animals were continuously observed during the first 30 minutes after dosing and observed during the first 4 hours up to 24 hours and subsequently observed daily for 14 days. Each animal was individually observed and recorded. Observations included; behavioral patterns, sleep patterns, changes in skin and fur, eyes, and mucous membranes. Careful attention was given to the observation of sleep, coma, convulsions, salivation, diarrhea, tremor, lethargy, and mortality. Changes in wellness parameters were compared with that of control animals.

1.3. Animals

Thirty male albino rats (Wistar strain) weighing between 150 to 200 g were purchased from the Animal Facility, Babcock University, and kept at room temperature for 24 hours, day and night. The rats were acclimatized for 2 weeks under laboratory conditions before the experiment. The animals were housed in polypropylene cages lined with wood shavings and provided with a commercial pellet diet from Ladokun feeds, Ibadan, Oyo state Nigeria and water *ad libitum.* Animals were humanely handled and maintained following the National Institute of Health Animal Care and Use Guidelines (NIH, 2011) and Babcock University Health Research Ethics Committee clearance was obtained with certificate number BUHRC505/19.

1.4. Experimental animal design

The animals were randomly assigned into six (6) experimental groups (I - VI) of five (5) rats each. Rats were orally administered with different doses of 2, 3-dimethyl naphthalene, and 10 mg/kg *b.w.* diclofenac sodium, using corn oil as a vehicle in each of the experimental models.

1.5. Carrageenan-induced rat paw edema model

Paw edema was induced in rats following the modified method described by Liao *et al.* (1993). Six groups of five (5) rats per group were used in this study. Group 1 served as a negative control (normal group), group II

served as a positive control (control untreated), group III received diclofenac sodium 10 mg/kg per oral (reference drug) and group IV-VI were given 2,3-dimethyl naphthalene at 5, 10 and 15 mg/kg *b.w*, respectively. Carrageenan (0.1 mL of 1% prepared as a suspension in distilled water) was injected into the sub-plantar tissue of the left hind paw of the rat 30 minutes after treatment. The volume of the resulting paw edema was measured at zero (0), half an hour, and a 1-hour interval for 6 hours using a micrometer screw gauge. The percentage inhibition at each time interval was calculated as follows:

$$\%inhibition = [(Ct - C0)control - (Ct - C0)treated \div (Ct - C0)control] \times 100$$

C_0 = paw size before carrageenan injection

C_t = paw size after carrageenan injection

1.6. Cotton Pellets-Induced Granuloma

The anti-granuloma activity of 2,3-dimethyl naphthalene was evaluated using cotton pellets-induced granuloma according to the method described by Winter and Porter (1957). The rats were divided into six experimental groups of five rats each (n =5), Group 1 served as a negative control (normal group), group II served as a positive control (control untreated), group III received diclofenac sodium 10 mg/kg *b.w* (reference drug) and the group IV-VI - were given 2,3-dimethyl naphthalene at 5, 10 and 15 mg/kg *b.w*, respectively. Thirty minutes after drug administration, an autoclaved cotton pellet (50 ± 1.0 mg) was aseptically implanted subcutaneously into the back region of each rat under anesthesia (75 mg/kg *b.w* ketamine). Treatment was done once daily for 7 days. On the 8th day, the animals were anesthetized again and cotton pellets were surgically removed, freed from the extraneous tissue, and then dried in a hot-air oven overnight at 60°C. The constant weight of the dried pellets was taken and the increment in the dry weight of the pellets was recorded as a measure of granuloma formation. The percentage inhibition of granuloma tissue development was determined (Winter & Porter 1957):

$$\%inhibition = \left[\left(weight\ of\ pellet(control) - weight\ of\ pellet\ (treated)\right) \div weight\ of\ pellet(control)\right] \times 100$$

Statistical Analysis

The difference between means was determined using Analysis of Variance (ANOVA) and data are expressed as mean ± standard error of mean (SEM) with significance level set at $p< 0.05$

3.0. Results

3.1. Wellness parameters analysis

Wellness analysis showed that the behavioral patterns, skin, fur, eyes, mucous membrane, and sleep pattern of the animals treated with 2,3-dimethyl naphthalene and the control untreated animals were found to be similar. This study showed that there was no recorded

mortality in the animals administered orally with 100 and 320 mg/kg *b.w.* doses of 2,3-dimethyl naphthalene.

3.2.Effect of 2,3-dimethyl naphthalene on carrageenan-induced rat paw edema

Data in Figure 1 showed that diclofenac sodium (10 mg/kg *b.w)*, and 2,3-dimethyl naphthalene at 5, 10 and 15 mg/kg *b.w.* treated groups had significantly (p <0.05) reduced paw edema by 1.07 ± 0.22 mm (81.50%), 1.71 ± 0.36 mm (55.82%), 2.17 ± 0.45 mm (21.49%) and 2.23 ± 0.39 mm (14.38%), respectively when compared with the control untreated group having paw thickness of 2.51 ± 0.68 mm (0%). Diclofenac sodium significantly (p <0.05) reduced paw edema in rats compared with the doses of 2,3-dimethyl naphthalene at the end of 6 hours.

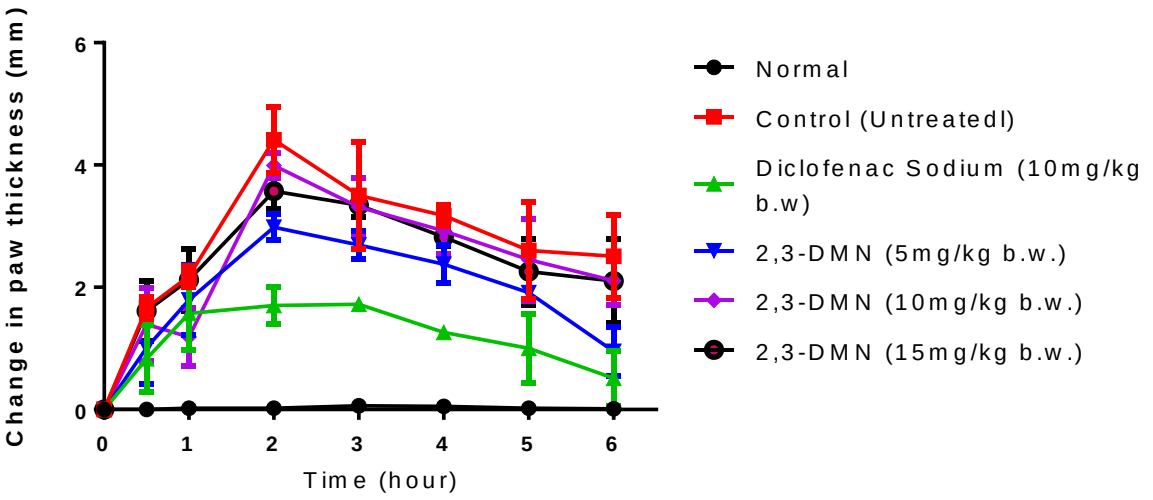

Figure 1. Effects of different doses of 2,3-dimethyl naphthalene and diclofenac sodium on paw edema of rat induced with inflammation using carrageenan

2,3-DMN indicates 2,3-dimethyl naphthalene, *b.w* indicates body weight, Normal indicates not induced and not treated, Untreated indicates induced and not treated.

3.2. Anti-inflammatory effect of 2,3-dimethyl naphthalene on cotton pellet-induced granuloma

Data in Figure 2 showed that animals treated with 10 mg/kg *b.w* diclofenac sodium (22.60 ± 2.83 mg [59.28%]) and 2,3-dimethylnaphthalene at 5 mg/kg *b.w* (55.65 ± 3.94 mg [11.48%]), 10 mg/kg *b.w.* (38.65 ± 4.70 mg [38.52%]) and 15 mg/kg *b.w* (37.93 ± 3.47 mg [39.69%]) had significantly (p < 0.05) inhibited cotton pellets-induced granuloma when compared with control untreated group. More so, diclofenac sodium treated group had a significantly (p<0.05) high anti-granuloma activity, when compared with 2,3-dimethylnaphthalene at 5 mg/kg, 10 mg/kg and 15 mg/kg

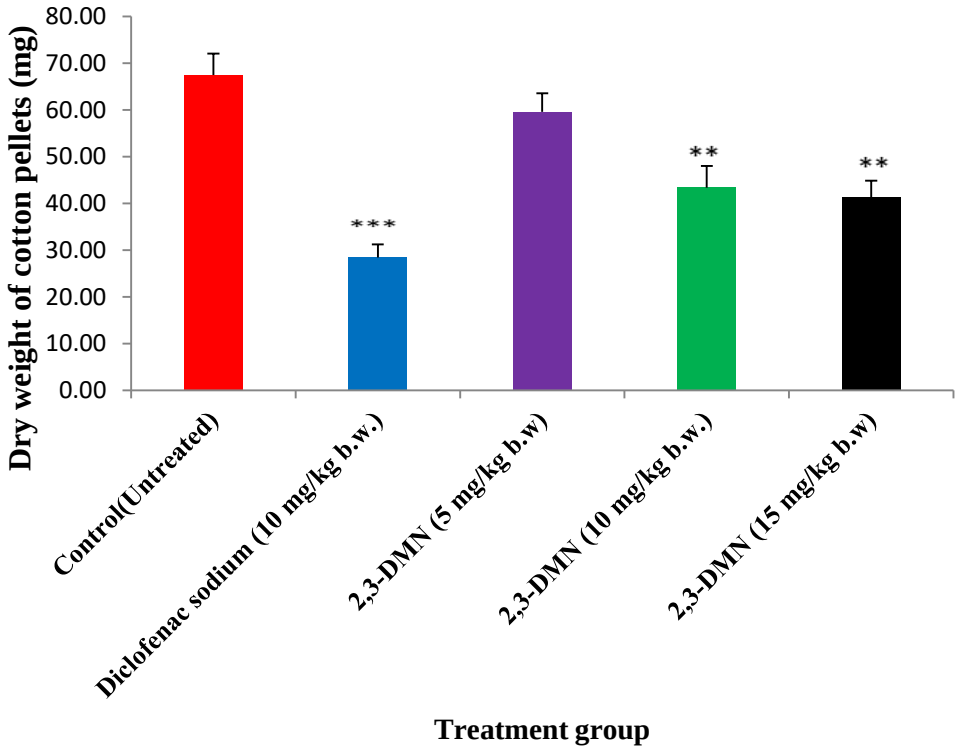

Figure 2 Effects of 2,3-dimethyl naphthalene on cotton pellet-induced granuloma

** and *** indicates significant difference at $p < 0.05$, 2,3-DMN = 2,3-dimethylnaphthalene, control (untreated) indicates induced and not treated

Discussion

Inflammation has been associated with numerous severe disorders including rheumatoid arthritis, osteoarthritis, cardiovascular diseases, and autoimmune diseases (Chen *et al.*, 2018). Pharmaceutical drug discovery and development are targeted towards researching and synthesizing novel anti-inflammatory agents including drugs containing naphthalene and its derivatives such as β-naphthol, nafcillin, and tolnaftate have been applied in pharmaceutical drug development (Pandya *et al.*, 2012). However, 2,3-dimethyl naphthalene has not been investigated for its anti-inflammatory activity.

In this study, oral administration of 2,3-dimethyl naphthalene wellness parameters showed no apparent signs of toxicity and mortality at 320 mg/kg *b.w.* which suggests that 2,3-dimethyl naphthalene could be safe at this level. Evaluation of the *in vivo* anti-inflammatory activity of 2,3-dimethyl naphthalene using different inflammation models showed that it suppressed carrageenan-induced paw edema in the second hour of induction in a dose-dependent manner, which suggests that it could possess anti-inflammatory activity. This finding appears to support the previous claim that naphthalene derivatives are potential anti-inflammatory

agents (Pandya *et al.,* 2012; Patel & Patel, 2019).

The carrageenan-induced inflammation model is an acute and sensitive model widely used to evaluate the anti-edematous activity of drugs (Kalpesh *et al.,* 2019). Carrageenan-induced edema is believed to be a biphasic response that involves the release of pro-inflammatory and inflammatory mediators such as histamine, serotonin, leukotriene, and bradykinin which affect vascular permeability (Amdeka *et al.,* 2012; Zhao *et al.,* 2018). The first phase begins with the release of serotonin, histamine, and bradykinin after few hours of induction (Amdeka *et al.,* 2012). Various studies have demonstrated that edema induction by carrageenan causes the release of inflammatory mediators of acute inflammation (Zhao *et al.*, 2018; Anyasor *et al*., 2019). The effectiveness of 2,3-dimethyl naphthalene at the low dose (5 mg/kg b.w) to prevent inflammation or reduce the progression of the inflammation process could be due to rapid absorption with preventive effect towards the damaging stimuli in the acute phase. The process of inflammation involves three phases associated with a different pattern (acute, immune response and chronic inflammation), hence, it could be proposed that the reduction of carrageenan-induced paw edema by 2,3-dimethyl naphthalene might be through the inhibition of the release of inflammatory mediators or inhibition of their action at the site of tissue injury.

It was also noted that 2,3-dimethyl naphthalene inhibited cotton pellets-induced granuloma in rats. This further supports the observation that naphthalene derivatives possess anti-inflammatory properties (Anyasor *et al.,* 2015). In cotton pellets-induced granuloma, granuloma development consist of two phases; a transudate and a proliferative, it occurs for several days after implantation of the sterilized cotton pellet (Subash *et al.,* 2016; Zhao *et al.,* 2018). The proliferative phase of inflammation involves the actions of neutrophils, macrophages, fibroblasts, and the activation of monocytes and lymphocytes, which are basic sources of granuloma formation (Zhao *et al.,* 2018). The monocyte-derived macrophages are then released at the site of injury for the elimination of the cotton pellet implant. However, the failure of the macrophages to eliminate the cotton pellet implant results in the release of interleukin-12 (IL-12) followed by T-lymphocytes activation, which releases interferon-γ (IFN-γ), thereby flooding the site of injury with other macrophages. The massive release of macrophages and T-lymphocytes results in the formation of a granuloma as the macrophages fuse (Hisamuddin *et al.,* 2019).

The extent of granulomatous tissue formed in this study is equivalent to the dried weight of the cotton pellets that are harvested while the wet weight of cotton pellets includes the transudate formed. Hence, the reduction of the dried weight of cotton pellets by 2,3-dimethyl naphthalene in a dose-dependent manner suggests that the proliferative phase could have been effectively suppressed and the granuloma formed reduced. It is conceived

that this phenomenon might have been due to inhibition of collagen, fibronectin, and glycosaminoglycan synthesis by 2,3-dimethyl naphthalene (Aishwarya *et al.*, 2018).

Conclusion

Findings from this study showed that 2,3-dimethyl naphthalene at 5, 10, and 15 mg/kg *b.w.* demonstrated anti-inflammatory activity although the highest activity was observed in the least dose. It also showed that its anti-inflammatory mechanism of action might be through inhibition of mediators of acute inflammation and pro-inflammatory signals such as inhibition of macrophage activation, infiltration, and aggregation.

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