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SHORT COMMUNICATION

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Anti-Inflammatory Activity of the Combined Ethanol Extracts of *Newbouldia laevis* and *Moringa oleifera* leaves

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Abstract: Researches have revealed the anti-inflammatory efficiencies of *Newbouldia laevis* and *Moringa oleifera* leaf extracts when used as monotherapies, however, there is dearth of information on the anti-inflammatory activities of the extracts when combined. Egg albumin was used to induce paw inflammation. Cotton thread method was used to measure linear paw circumference and reduction in paw size after treatment was the basis for determining anti-inflammatory activity. Rats were assigned into six groups of three animals each and inflammation was induced with a dose of 25mg kg^{-1} of egg albumin. Groups 1 and 2 were treated with 25mg/kg body weight of *N. laevis* and *M. oleifera* extracts respectively, while groups 3, 4 and 5 were administered combined doses of *N. laevis* and *M. oleifera* extracts in ratios 1:1, 2:1 and 1:2 respectively. Group 6 was administered 25mg/kg body weight of the reference drug, aspirin to serve as positive control. Groups 1 and 2 showed percentage mean inflammation inhibition of 25.3 % and 53.3% respectively. Animals in groups 3, 4 and 5 showed 41.8%, 29.7% and 48.1% percentage mean inhibition of inflammation respectively after five hours of treatment. The highest activity was found to be in the group treated with *M. oleifera* extracts alone (53.3%), followed by the group treated with dose ratio 1:2 of *N. laevis* and *M. oleifera* (48.1%). However, there was no significant difference (at $p<0.05$) between these two groups. The results obtained in this study show that though monotherapy with *Moringa oleifera* gave the best anti- inflammatory activity, combination of the two extracts could still be used to elicit a cumulative anti-inflammatory activity.

KEYWORDS: *Newbouldia laevis*, *Moringa oleifera*, Egg-albumin, Paw inflammation

1.0 Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al.*, 2007). Inflammation is not the same as infection, even in cases where inflammation is caused by infection (Abbas and Lichtman, 2009). The five principal signs of inflammation are hotness (calor), redness (dolor) due to increased blood flow to the area (redness), swelling (inflammation), loss of function (due to accumulation of exudates), and pain (Venkatesh *et al.*, 2008). When cells are injured, the mast cells signal inflammatory response, releasing NF kappa B, the key regulator of the inflammatory response system. This results in the expression of several pro-inflammatory proteins such as COX2 and iNOS that causes pain, fever, swelling and heat of an affected area. Simultaneously, a series of pro-inflammatory cytokines such as interleukine-2, tumour necrosis factor-alpha, and interferon gamma are released. Inflammation gradually subside once the cause of the injury has been eliminated or the integrity of the living tissue has been protected, however, severe inflammation may lead to oxidative stress induced by harmful substances such as reactive oxygen species, neutrophil-derived free radicals and reactive nitrogen species produced by neutrophils and macrophages during inflammation (Valko *et al.*, 2006). In chronic conditions, inflammation is usually persistent but low grade, with occasional acute aggravations (Venkatesh *et al.*, 2008). Non-steroidal (aspirin, ibuprofen, naproxen) and steroidal (cyclocort, diprosone, decadron,

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locorten, flonase, etc) are currently used today for the treatment of inflammation, however, as a result of the inbuilt problems related with the non-steroidal (such as gastrointestinal consequences, fluid retention, nephropathies, skin rashes, hepatitis, interaction with drugs such as antihyperglycemic and antihypertensive agents (Hudson and Hawkey, 1993; Russell, 2001; Hawkey and Langman, 2003) as well as steroidal anti-inflammatory drugs (such as altered response to physical stress, steroid withdrawal syndrome, infection, osteoporosis, weight gain, insomnia, mood changes, elevated blood pressure and sugar, eye problems, aseptic necrosis, to mention a few (Fields, 2009), there is constant search especially from natural sources for alternative agents (Akah *et al.*, 2003). Clinical experiments have validated the effectiveness and safety of some plants used traditionally to manage pain and inflammation (Musa *et al.*, 2007), hence, there is a constant demand for better therapeutic alternatives. Examples of medicinal plants that have been investigated for their individual use as anti-inflammatory agent are *Newbouldia laevis* and *Moringa oleifera.*

Newbouldia laevis commonly known as boundary tree is a medium sized angiosperm of the *Bignoniaceae* family. It is native to tropical Africa and grows from Guinea Savannah to dense forest (Arbonnier, 2004). In Nigeria, the plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as a vermifuge to round worms (Usman and Osuji, 2007). The root, leaf, stem and fruits have been used variously for febrifuge; wound dressing and stomach ache (Iwu, 2000), including inflamed sores, ulcers and abscesses. A study has also shown that a 95% ethanol leaf extract of *N. laevis* reduced the paw edema induced by fresh egg albumin and was significantly more potent than indomethacin. A study carried out by Usman *et al* (2008), showed that there was a dose dependent inhibition of inflammation in rats, by ethanol extract of *N. laevis* flower.

Moringa oleifera is of the family *Moringaceae*. It is commonly known as drumstick. It is a very popular backyard tree that grows to over 9 m height. It has soft, white corky trunk and branches bearing a gummy bark. The flowers, tender leaves and pods are eaten as vegetable. The leaves are rich in iron and therefore highly recommended for expectant mothers. Since all essential amino acids are present, *M. oleifera* may be rightly called a complete food for total nutrition. The whole *M. oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac (Nadakami, 1973).

The aqueous extracts of roots and barks of *M. oleifera* were found to be effective in preventing implantation, (Shukla and Mathur, 1988) whereas the aqueous extracts of its fruits have shown significant anti-inflammatory activity. Methanolic extracts of its leaves have shown anti-ulcer activity while ethanolic extracts of the seeds have exhibited anti-tumour activity (Guevara *et al.*, 1999). The methanol root extract of *M. oleifera* was found to be effective in both acute and chronic inflammation and inhibited both cell and fluid accumulation (Ezeamuzie *et al.*, 1996). In a recent study, 50%ethanolic leaf extract of *M. oleifera* was found to exhibit significant anti-inflammatory activity by inhibiting the edema induced after the injection of carrageenan into the hind paw of rats (Verma *et al.*, 2014). Also, there was significant inhibition of acetic acid-induced increased vascular permeability in mice (Verma *et al.*, 2014).

However, there is no scientific report validating the medicinal uses of the combined plants of *N. laevis* and *M. oleifera* in the treatment of inflammation. This led to the investigation of the anti-inflammatory effect of the combined ethanolic leaf extracts of *N. laevis* and *M. oleifera* in rats to evaluate their combined anti-inflammatory activity using fresh egg-albumin induced hind paw oedema in rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Materials

The leaves of the plants, *Newbouldia laevis and Moringa oleifera* were collected from Bosso community in Minna, Niger State, Nigeria.

2.1.2 Animals

Thirty albino male rats with average weight of 200 - 280g were purchased from the Animal Facility Centre, Benue State University, Nigeria.

2.1.3 Inflammatory Agent

The inflammatory agent used in this study was fresh undiluted chicken egg albumin

2.2 Methods

2.2.1 Preparation of Extract

The powdered plant samples (50 g) were each extracted in 95% (v/v) of ethanol (300 ml) using reflux extractor at 70°C for 2 hours. Thereafter, they were filtered into beakers using filter papers and glass funnel. The extracts were concentrated using a rotary evaporator at 70°C to obtain a greenish sticky extracts [yield: 13.4% (NL), 8.75 %(MO)]. NL: *Newbouldia laevis*, MO: *Moringa oleifera*.

2.2.2. Anti-inflammatory Study

The rats were assigned into seven groups of three animals each. The experimental animals were administered with a dose of 25 mg kg^{-1} (intraperitoneally) of the extracts and the reference drug (aspirin). Groups 1 and 2 were administered *N. laevis* and *M. oleifera* respectively, groups 3, 4, and 5 were administered combined extracts of *N. laevis*, MO: *M. oleifera* in the ratio 1:1, 2:1, and 1:2 respectively. Group 6 was administered the reference anti-inflammatory drug (aspirin) and group 7 served as the negative control, which received distilled water. Acute inflammation was induced by injecting 0.1 ml of freshly prepared egg albumin into the sub-plantar region of the right hind paw of the rats (Ojewole, 2006). The rats developed inflammation after 30 minutes of induction with the egg albumin. The linear paw circumference/diameter was measured using the cotton thread method (Sofidiya *et al.*, 2010) before induction and at 1 hour interval for 5 hours, after the administration of anti-inflammatory agents (extracts and reference drug).

Some parameters were set after the injection of the inflammatory agent and were recorded at different time intervals throughout the experimental procedures, to serve as basis for assessment of anti-inflammatory effect of extracts. These parameters were:

0: rats walk normally.
1: partial elevation of paw on the floor.
2: elevation without contact to the floor.
3: licking, biting or shaking of paw.
4: rat remains in one position.
5: light bearing of the paw on the floor (Ojewole, 2006).

The difference between the readings at time 0 hours and different time interval was taken as the thickness of inflammation. The percentage inhibition of inflammation was calculated for each dose-ratio (25mg/kg b.w) at different hours using the following expression described by Tanko *et al* (2008).

$$\text{Inhibition (\%)} = \frac{\text{MPD (Control)} - \text{MPD (Treated)} \times 100}{\text{MPD}}$$

where:
MPD = Mean paw diameter

2.2.3 Data Analysis

Results were expressed as mean ± Standard Error of Mean. The data was statistically analysed using one-way Analysis of Variance. Results were statistically significant at p less than 0.05 ($p < 0.05$).

3.0 Results

The effect of combined ethanol extracts of *N. laevis and M. oleifera* leaves on egg albumin - induced paw inflammation of experimental animals at different time intervals are shown in Table 1. Results in the treated groups with plants' extracts and the reference drug were significantly different from those in the corresponding control group. The group treated with 2:1 ratio of ethanol extract of *M. oleifera* and *N. laevis* gave the best result when compared within the extracts treated groups, though not significantly different when

compared with the group treated with the reference drug.

Table 2 shows the percentage inhibition of paw oedema in the experimental animals both in the groups treated with the plants' extracts and the group treated with the reference drug (aspirin). At the third hour, there was an increase in the percentage inhibition of inflammation in the group treated with both plants' extracts and the reference drug aspirin. The group treated with the standard drug and *M. oleifera* extract alone exhibited the highest percentage mean of inhibition after five hours.

4.0 Discussion

Results obtained from the present study have demonstrated the ability of combined ethanol extracts of *N. laevis* and *M. oleifera* leaves to reduce inflammation induced by egg albumin. The reduction produced by the combination was significant and ratio-dependent (Table 2). The highest activity was found to be in the group treated with *M. oleifera* extracts alone (53.3% reduction in paw size). The appreciable % inhibitory activity shown by the ethanol extract of *M. oleifera*, is in line with a recent study, where 50% ethanolic leaf extract of *M. oleifera* was found to exhibit significant anti-inflammatory activity by inhibiting the edema induced by the injection of carrageenan into the hind paw of rats (Verma *et al.*, 2014). The group treated with dose ratio 1:2 of *N. laevis* and *M. oleifera* had a percentage inhibition of 48.1% reduction. The percentage reduction produced by the 1:2 combination when compared to that produced by *M. oleifera* alone gives credence to the fact that the dominance of *M. oleifera* extract in the combination (1:2) is responsible for the reduction of inflammation in the present study. The lowest percentage inhibitory activity was observed in group 1 (25.3%) treated with ethanol extract of *N. laevis* alone. This is a demonstration of lower anti-inflammatory activity by the ethanol extract of *N. laevis* leaves. It is therefore not expected to produce a positive synergistic action in combination because it is originally not very active. But there may be a likelihood of enhancing its activity at higher doses as demonstrated in a study carried out by Usman *et al.* (2008), which showed that there was a dose - dependent inhibition of inflammation in rats by ethanol extract of *N. laevis* flower used alone.

The ability of extracts to reduce inflammation induced in the rat paw by injection of an inflammatory agent is basis for ascertaining anti-inflammatory potential.

This study has succeeded in further ascertaining the potency of ethanol extract of *M. oleifera* leaf as an anti-inflammatory agent, while its combination with other plant extracts like *N. laevis* may affect its activity. The study has shown that it is not in all cases that combinations of plant extracts elicit positive synergy.

As for possible mechanism of action, it has been shown that three main mediators are responsible for acute and chronic inflammatory reactivities (Kasahara *et al.*, 2002). The first phase of inflammation is attributed to the release of histamine, serotonin or bradykinin by local cells. After few hours, there is release of prostaglandins (Morris, 2003). It is therefore possible that the extracts exhibited their anti-inflammatory activities by inhibiting the synthesis, release or action of inflammatory mediators including histamine, serotonin and prostaglandin known to mediate acute inflammation induced by inflammatory agents, which is likely also to involve egg albumin (Haiping *et al.*, 2008).

The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells (e.g. neutrophils, thus indicating that the extracts may also exert their anti-inflammatory effect partly through the inhibition of neutrophil infiltration (Dordevic *et al.*, 2007) and prostaglandins produced by tissue macrophages (Gupta *et al.*, 2006).

Conclusion

It could be concluded that, though monotherapy with ethanol extract of *Moringa oleifera* leaf gave the best anti-inflammatory activity, combination of the two extracts could still be used to elicit a cumulative synergistic anti-inflammatory activity.

Table 1: Effect of combined ethanol extracts of *Newbouldia laevis and Moringa oleifera leavis* on egg albumin-induced paw inflammation in rats

Drug/extract Dose ratio ($mgkg^{-1}$)	Paw odema volume/diameter (cm)				
	1h	2h	3h	4h	5h
Distilled water	0.47 ± 0.03^{b}	0.48 ± 0.02^{a}	0.42 ± 0.02^{a}	0.38 ± 0.02^{a}	0.32 ± 0.02^{a}
Aspirin 25mg/kg	0.35 ± 0.11^{c}	0.22 ± 0.06^{d}	0.18 ± 0.04^{ef}	0.12 ± 0.05^{f}	0.07 ± 0.03^{d}
ENL 25mg/kg	0.57 ± 0.03^{a}	0.37 ± 0.06^{b}	0.30 ± 0.06^{c}	0.30 ± 0.06^{b}	0.22 ± 0.04^{b}
EMO 25mg/kg	0.33 ± 0.12^{c}	0.22 ± 0.06^{d}	0.15 ± 0.05^{f}	0.15 ± 0.05^{ed}	0.13 ± 0.04^{c}
ENL:EMO 25mg/kg (1:1)	0.43 ± 0.07^{b}	0.32 ± 0.09^{c}	0.22 ± 0.04^{de}	0.17 ± 0.04^{d}	0.12 ± 0.04^{c}
ENL:EMO 25mg/kg (2:1)	0.45 ± 0.03^{b}	0.40 ± 0.03^{b}	0.35 ± 0.03^{b}	0.25 ± 0.03^{c}	0.15 ± 0.05^{c}
ENL:EMO 25mg/kg (1:2)	0.42 ± 0.09^{b}	0.32 ± 0.04^{c}	0.18 ± 0.03^{ef}	0.13 ± 0.03^{f}	0.08 ± 0.03^{d}

Each values are mean ± SEM; number of animals used (n=3). Values with the same superscript along the column are not significantly different from each other; values with different superscript are significantly different ($p < 0.05$)

Table 2: Percentage inhibition of paw oedema exhibited by ethanol extracts of *Newbouldia laevis* and *Moringa oleifera* leaves

Treatment	Percentage inhibition					
	1h	2h	3h	4h	5h	Mean % Inhibition
Aspirin	32.1	55.1	56.1	69.5	79.0	58.4
ENL	21.4	24.0	28.0	21.7	31.5	25.3
EMO	28.7	51.1	64.0	60.8	58.3	53.3
ENL:EMO (1:1)	7.3	34.4	48.0	56.4	63.1	41.8
ENL:EMO	14.4	30.4	32.1	34.7	36.1	41.3
ENL:EMO (1:2)	10.7	34.4	56.1	63.3	73.8	48.1

Each values are mean ± SEM; number of animals used (n=3). Values with the same superscript along the column are not significantly different from each other; values with different superscript are significantly different ($p < 0.05$); ENL: Ethanol extract of *Newbouldia laevis*; EMO: Ethanol extract of *Moringa oleifera*; ENL:EMO: Ethanol extract of *Newbouldia laevis* and Ethanol extract of *Moringa oleifera* extracts, (1:1); ENL:EMO: Ethanol extract of *Newbouldia laevis* and Ethanol extract of *Moringa oleifera* extracts (2:1); ENL:EMO: Ethanol extract of *Newbouldia laevis* and Ethanol extract of *Moringa oleifera* extracts (1:2); Aspirin: Standard drug; DW: Distilled water (Negative control).

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References

Abbas, A. B. and Lichtman, A. H. (2009). Ch.2 Innate Immunity. In: Saunders (Elsevier). Basic Immunology. Functions and disorders of the immune system. pp. 369 – 376.

Akah, P. A., Mokoli, C. O. and Nwafor, S. V. (2003). Anti-inflammatory activity of plants Natural remedies. Journal of Natural Remedies 3(1): 01 - 30.

Arbonnier, M. (2004). *Trees, Shrubs and Lianas of West African Dry Zones*. Cote d'Ivorie: CIRAD, Margraf Publishers GMBH MNHN, p. 194.

Dordevic, S., Petrovi´c, S., Dobri´c, S., Milenkovi´c, M., Vu˘ci´cevi´c, D., Zi˘zi´c, S. and Kuki, J. (2007). Anti-microbial, anti-inflammatory, anti-ulcer and anti-oxidant activities of *Carlinaac anthifolia* root essential oil. Journal of Ethnopharmacology 109: 458-463.

Ezeamuzie, I. C., Ambadederomo, A. W., Shode, F. O. and Ekwebelem, S. C. (1996). Antiinflammatory effects of *Moringa oleifera* root extract. International Journal of Pharmacognosy 34: 207 – 212.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S. and Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1betageneration. Clinical Experimental Immunology 147(2): 441-449.

Fields, R. (2009). *Steroid side effects: How to reduce Corticosteroid side effects*. Attending Physician, Hospital for special surgery, Professor of Clinical Medicine, Weill Cornell Medical College.

Guevara, A. P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H. and Nishino, H. (1999). An antitumor promoter from *Moringa oleifera* Lam. Journal of Mutatation Research 44: 181 – 188.

Gupta, M., Mazumder, U. K., Gomathi, P. and Thamilselvan, V. (2006). Anti-inflammatory evaluation of leaves of *Plumeria acuminata*. BMC Complementary and alternative medicine 6(36): 1472-6882.

Hawkey, C. J. and Langman, M. J. S. (2003). Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles of COX-2 inhibitors and proton pump inhibitors. British Medical Journal 52(4): 600-608.

Haiping, L. I., Xiaoxiang, L. U., Shuhai, Z, Meijun, L. U. and Hongmei, L. (2008). Anti-Inflammatory activity of Polysaccharide from *Pholiota nameko*. Biochemistry (Moscow) 73(6): 669-675.

Hudson, N. and Hawkey, C. J. (1993). Non-steroidal anti-inflammatory drugs associated upper gastrointestinal ulceration and complications. European Journal of Gastroenterology and Hepatology 5: 412-419.

Iwu, M. M. (2000). *Handbook of African Medicinal plants*. CRC press, Inc., London. pp. 19-23.

Kasahara, Y. K., Yasukawa, S., Kitanaka, M., Taufiq, K. and Evans, F. J. (2002). Effect of methanol extract from leave petals of *Tagestes patella* L. on acute and chronic inflammation model. Phytotherapy Research 16: 217-222.

Morris, C. J. (2003). Carrageenan-induced paw inflammation in rat and mouse. *Methods in Molecular Biology* 225: 115-121.

Musa, Y. M.., Haruna, A. H., Yaro, A. A., Ahmada, H. and Usman, (2007). Analgesic and inflammatory effects of leaf extracts *pseudocedrella kotschyii* harms (Meliaceae). Journal of Pharmacology and Toxicology 2: 542-550.

Nadakarni, A. K. (1973). *Indian Meteria Medica*. Mumbai, Popular Prakashan, pp. 72.

Ojewole, J. A. (2006). Analgesic, anti-inflammatory and hypoglycaemic effects of ethanol extract of *Zingiberofficinale* (Roscoe) rhizomes (*Zingiberaceae*) in mice and rats. Phytotherapy Research 20: 764-772.

Russell, R. I. (2001). Non-steroidal anti-inflammatory drugs and gastrointestinal damage-problems and solutions. Postgraduate Medical Journal 77: 82-88.

Sofidiya, M. O., Odukoya, O. A., Adedapo, A. A., Mbagwu, H. O. C., Afolayan, A. J. and Familoni, O. B. (2010). Investigation of the anti-inflammatory and anti-nociceptive activities of *Hymenocardia acida* Tul. (*Hymenocardiaceae*). African Journal of Biotechnology 9(49): 8454-8459.

Shukla, S. and Mathur, R. (1988). Prakash Antifertility profile of the aqueous extract of *Moringa oleifera* roots. Journal of Ethnopharmacology 22(1): 51-62.

Tanko, Y., Kamba, B., Saleh, M. A., Musa, K. Y. & Mohammed, A. (2008). Anti-nociceptive and anti-inflammatory activities of ethanolic leave extract of *Newbouldia laevis* in mice and rats. International Journal of Applied Research in Natural Products 1(3): 13-19.

Usman, H. and Osuji, J. C. (2007). Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis*. African Journal of

Traditional, Complementary and Alternative Medicine 4(4): 476 – 480.

Usman, H., Yaro, A. H. and Garba, M. M. (2008). Analgesic and anti-Inflammatory screening of *Newbouldia laevis* Flower in Rodents. Trends in Medical Research 3: 10-15.

Valko, M., Rhodes, C. J., Momol, M. I. and Mazuri, M. (2006). Free radical metals and anti-oxidants in oxidative stress induced cancer. Chemico-Biological Interaction 160: 1-40.

Venkatesh, T., Nandini, Y., Sai, L., Kishore, V., Satish, K., Archana, P. and Malathi, G. (2008). Pharmacological screening of anti-inflammatory activity of *Ksheerabala thailam*'. https://www.pharmatutor.org/articles/ksheerabala-thilam-pharmacological-activity

Verma, V. K., Hussain, Z. & Verma, M. (2014). Antiinflammatory and Antinociceptive activity of *Moringa oleifera*. Journal of Biomedical and Pharmaceutical Research 3(1): 14-21.