

PHYSICOCHEMICAL, ANTIMICROBIAL AND ANTI-INFLAMMATORY EVALUATION OF FIXED OIL FROM BOA CONSTRICTOR

ABIODUN FALODUN*^a, OMONKHELIN JOSEPHINE OWOLABI^b, and OBASUYI OSAHON^c

Departments of ^a Pharmaceutical Chemistry ^b Pharmacology and Toxicology, ^c Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Abstract: Boa constrictor is one of the snakes found in the riverine areas of Nigeria, especially in the Niger Delta regions. The fat obtained from the snake is used ethno-medicinally for the treatment of burns and inflammatory conditions. The purpose of this study was to validate the traditional use of this crude fat and oil. The fat obtained from the Boa snake was subjected to some physicochemical screening tests. A systematic chemical and antimicrobial investigation was carried out using some bacterial found in wound such as *Staphylococcus aureus*, *B. subtilis* and *Streptococcus pyogenes*. The degree of zone of inhibition was a measure of the antimicrobial activity of the fat and oil. The maximal inhibitory dilution was determined for significant zone. The anti-inflammatory investigation was done using the croton oil induced ear edema. The results of the study revealed a potent anti-inflammatory and a significant antimicrobial activity of the fat from Boa constrictor against *S. aureus* and *S. pyogenes* organisms, thus, justifying the traditional usage of the fat of Boa constrictor.

Keywords: Boa snake, fat, antimicrobial activity, anti-inflammatory, chemical properties.

There are various approaches to the selection of plant species for study as potential drug sources (1, 2). However, the most popular approach is through ethno-medicine and one of the most fascinating approaches is through field study of wildlife termed zoocognosy (3).

Narrowing the animal kingdom down to reptiles, the snakes have also proven to be useful sources of medicine. The angiotensin converting enzyme inhibitor Captopril was first isolated from the venom of the Brazilian arrowhead viper (4). Ancrod, an anticoagulant which destroys fibrinogen, hence, depleting the blood of its essential factor for fibrin formation was obtained from the venom of the Malaysian pit viper (5). The fat extracted from under the skin of Boa constrictor snake has been used for many years as an anti-inflammatory agent.

Boa constrictor is a cold-blooded animal found in tropical rainforest area, desert and grassland (6). Depending on their sex (females are larger) and subspecies, they can reach from 5 to 12 feet as adults. Boa fat originally came from China where it was used as a remedy for inflammation and pain in rheumatoid arthritis, bursitis and other similar condition. It is still used as a pain reliever in China (7).

It is also used locally in Nigeria (especially in the Southern and Western parts) for the treatment of burns and inflammatory conditions, and these necessitated our present research work.

EXPERIMENTAL

Animal Materials:

Boa fat: The globule of the boa fat was obtained from Oba market, Ring road, Benin-city and it was authenticated by the farmer who supplied the fat as fat from a Boa constrictor.

Preparation of Boa oil: The boa fat was placed in a conical flask and melted in a water bath at 25°C until all the fat melted into oil from which different concentrations were prepared as required.

Laboratory Animals: Albino mice 20-40 g of both sexes were obtained from the laboratory animal centre, Ambrose Ali University, Ekpoma, Edo State, Nigeria. The animals maintained under standard environmental conditions had free access to diet (Bendel feeds, Ewu, Nigeria) and water *ad libitum*. All animals were acclimatized for two weeks.

Ethical approval

Approval for use of animals in this work was obtained from the Ethical Committee on the use of Animals for Experiments of the Faculty of Pharmacy, University of Benin, Benin City.

Drugs and Reagents

Croton oil and Tween 80 were purchased from Sigma-Aldrich Inc. USA. Hydrocortisone cream (Monic-Tee pharmacy, UBTH, Benin-city), diethyl

* Correspondence: e-mail: faloabi25@yahoo.com Phone: +2348032396550.

ether, chloroform and potassium hydroxide (BDH Chemicals, England).

Chemical evaluation

The boa constrictor fat was subjected to determination of some physicochemical properties such as saponification, acid, ester values and specific gravity, using standard methods (8-10).

Anti-microbial evaluation

The agar diffusion method described by Verpoorte, (11) was used. Inocula of test organisms obtained from source were prepared by growing each pure isolate in nutrient broth (Oxoid cm 31) for 18 hours at 30°C the overnight culture was matched with Macfarland turbidity standard to give approximately 10^6 cfu/mL. This was poured into sterile Petri dishes and used for analysis. The oil was tested at neat concentration this was delivered into wells (8 mm in diameter) bored into the surface of Mueller Hinton agar plates. Commercial discs containing amoxicillin 25 µg and ciprofloxacin 5 µg (Silva Hills Pharmaceuticals) were used in parallel in the agar diffusion method.

Staphylococcus aureus (NCTC 10788) maintained in our laboratory was set up along with the test organisms as a check on the effect of media and inherent sensitivity of isolates on zones of inhibition produced by the antibacterial substances. The fungal isolate (*Candida albicans*) was treated in a slightly different manner. It was first grown on Sabouraud Dextrose broth (oxoid cm 41) and assayed using Sabouraud dextrose agar. The plates were incubated at 37°C for 24 h, while the fungi were incubated at 25°C for 48 h. After incubation the zones of inhibition were recorded in millimeters (mm).

Determination of maximal inhibitory dilution (MID)

The test tube serial dilution methods of Bailey and Elvyn (12) and Greenwood (13) was used to determine the MID. Appropriate dilutions were made with dimethylsulfoxide (DMSO) and incorporated in Mueller Hinton broth to give a final volume of 1 mL in the tubes. One drop equivalent to 0.02 mL of organisms (prepared as previously described) was added to each test tube. The tests were incubated at 37°C for 24 h. The contents of each tube was streaked on a Mueller – Hinton agar plate and further incubated for 18 h to determine the MID. The maximal inhibitory dilution (MID) was recorded as the lowest concentration (highest dilution) of extract that inhibited visible growth.

Anti-inflammatory evaluation

Croton oil-induced ear edema

A modification of the (14) was used. Albino mice were allotted to groups of five mice each. The mice were treated with boa fat (different concentrations) and hydrocortisone cream (0.75 mg/ear).

Thirty minutes after treatment with boa fat or hydrocortisone cream, edema was induced in each mouse by applying a solution of croton oil in acetone (2 µg/20 µL) on the inner surface of the right ear. Three hours later, the mice were sacrificed by anesthesia using ether, then cervical dislocation and both ears cut off and weighed. Edema was determined by measuring the difference in weight between the right and left ears for each mouse.

Statistical analysis

Results are expressed as the mean \pm SEM. Statistical analysis of the data was done using Student's t-test and the results were considered significant when p value < 0.05.

RESULTS AND DISCUSSION

The acid value indicates the amount of fatty acids present in the fat, which in turn indicates the degree to which the fat can rancidify. As fats undergo rancidity, triglycerides are converted to fatty acids and glycerol, causing an increase in acid value. rancidification can be reduced by storing fats and oils in a place with little exposure to oxygen, low temperature and away from light, since light and heat accelerates the rate of reaction of fat with oxygen. The physicochemical properties are used in establishing standards for drugs and compounds. The iodine value of 133 is indicative of the high degree of unsaturation of the fat (i.e. the presence of conjugated bonds) and this is typical of long chain fatty acids. It was observed that the degree of rancidity was low.

Saponification value is a measure of the average molecular weight (or chain length) of all the fatty acids present in the fat. Saponification value of 312.69 indicates that the fat has a saponification equivalent of 179.42 indicating that 179.42 g of the fat will be saponified by 52.104 g of KOH i.e going by the relationship (saponification value) (saponification equivalent = 56,104). This gives an indication of the amount of moles of KOH that will be required in the complete saponification of the fat.

Table 2 shows that the neat concentration of Boa constrictor fat has antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and

Streptococcus, which are all Gram-positive organisms. No activity was shown against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Candida albicans*.

The maximal inhibitory dilution is shown in Table 3. The maximal inhibitory dilution (MID) was 1:8 for *Bacillus subtilis* and 1:16 for *Staphylococcus aureus* and *Streptococcus viridans*. Comparative analysis of the activity of substance against some commercially obtained antibiotics is revealed in Table 4. The activity of *Boa constrictor* fat was sim-

ilar to the activity of 25 µg of amoxicillin in *Streptococcus* spp.

The fact that the fixed oil from *Boa constrictor* produced zones of inhibition against Gram positive organisms such as *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus* spp indicate the presence of antibacterial activity which influences its use as anti-infective especially by the local populace for the treatment of burns and wound. However, not having activity against Gram negative organisms like *Escherichia coli*, *P. aeruginosa* and *Enterobacter aerogenes* indicates its narrow spectrum of activity. This may be due to the presence of hydrophilic substance in the cell wall of the Gram negatives. The extract showed no activity against *Candida albicans* which was the only fungus tested.

The performance of *Boa* fat against sensitive bacteria isolates did not show significant differences when compared with antibiotics discs – prepared with amoxicillin (Table 4).

Inflammation is an overlapping succession of changes, which occur due to tissue injury. Anti-inflammatory effects may be produced by a wide variety of chemical agents and there is no correla-

Table 1. Physicochemical characteristics of the oils of *Boa constrictor*.

| Parameter at 25°C | Value |
|----------------------------------|--------|
| Specific gravity | 0.81 |
| Acid value (mg KOH/g) | 310.73 |
| Ester value (mg KOH/g) | 3.92 |
| Saponification value (mg KOH/g) | 312.69 |
| Saponification equivalent | 179.42 |
| Iodine value (gram iodine/100 g) | 133.00 |

Table 2. Mean zone diameter of isolates from extract of *boa* fat.

| Organism | Mean zone diameter (mm)* |
|-------------------------------|--------------------------|
| <i>S. aureus</i> | 14 |
| <i>B. subtilis</i> | 14 |
| <i>Streptococcus spp</i> | 16 |
| <i>E. coli</i> | - |
| <i>P. aeruginosa</i> | - |
| <i>Enterobacter aerogenes</i> | - |
| <i>Candida albicans</i> | - |

* indicates the mean of 5 isolates; – no activity. Zone measuring ≥ 10 mm were acceptable as sensitive

Table 3. Maximal inhibitory dilution (MID) of *boa* fat.

| Organism | Mean zone diameter (mm)* |
|-------------------------------|--------------------------|
| <i>S. aureus</i> | 1/16 |
| <i>B. subtilis</i> | 1/8 |
| <i>Streptococcus spp</i> | 1/16 |
| <i>Escherichia coli</i> | N.T |
| <i>Pseudomonas aeruginosa</i> | N.T |
| <i>Enterobacter aerogenes</i> | N.T |
| <i>Candida albicans</i> | N.T |

N.T – not tested since there was no zone of inhibition.

Table 4. Comparison of the activity of commercial antibiotics against *boa* fat.

| Organisms | Mean zone <i>boa</i> fat | Diameter (mm) AM (25 mg) | Diameter (mm) CIP (5 mg) |
|-------------------------------|--------------------------|-----------------------------|-----------------------------|
| <i>S. aureus</i> | 14 | 15 | 33 |
| <i>B. subtilis</i> | 14 | 14 | 28 |
| <i>Streptococcus spp</i> | 16 | 16 | 30 |
| <i>Escherichia coli</i> | - | 17 | 24 |
| <i>Pseudomonas aeruginosa</i> | - | - | 28 |
| <i>Enterobacter aerogenes</i> | - | 20 | 28 |
| <i>Candida albicans</i> | - | N.T | N.T |

N.T – not tested, AM – Amoxicillin, CIP – Ciprofloxacin

Table 5. Effect of boa fat on croton oil-induced ear edema.

| Group | Dose | Mean difference | p value | Level of significance |
|---------------------------|-------------|-----------------|---------|-----------------------|
| Control | | 0.038 ± 0.006 | - | - |
| Standard (hydrocortisone) | 0.75 mg/ear | 0.0083 ± 0.001 | 0.0005 | Extremely significant |
| Boa fat | 12 mg/ear | 0.013 ± 0.004 | 0.0197 | Significant |
| | 6 mg/ear | 0.022 ± 0.001 | 0.0973 | Not significant |
| | 3 mg/ear | 0.024 ± 0.005 | 0.2404 | Not significant |

Values are the mean ± SEM of results obtained from 5 mice in each group

tion between their pharmacological activity and chemical structure.

An acute model of inflammation was used to investigate the possible anti-inflammatory activity of boa fat. The results obtained showed a dose-dependent anti-inflammatory effect on the model used (Table 5). The dose of 400 mg/kg produced the highest and significant anti-inflammatory activity.

The fat showed a significant activity on the croton oil-induced ear edema comparable to the effect of hydrocortisone. The croton oil-induced ear edema model in mice is useful for the evaluation of anti-inflammatory effects of topical steroids or non-steroidal anti-inflammatory agents. This model has the advantages of local response that involves the skin of the ear, topical application which avoids drug metabolism, also, the model uses very small amounts of drug which avoids wastage.

CONCLUSION

The result of the present study showed that the fat from the snake possessed antibacterial actions due to the inhibitory effect on commonly implicated bacteriae in burns.

The ability of boa fat to inhibit the inflammatory responses produced in the croton oil-induced ear edema in the mice showed that it possesses anti-inflammatory property which is probably mediated via inhibition of prostaglandins synthesis, also this mechanism may be responsible for the reason boa fat is used as an analgesic, since prostaglandins are also implicated in the pain production. However, further work will be needed to confirm the mechanism(s) by which it produces its anti-inflammatory effect as well as identify, isolate and characterize the active chemical constituent which could be added to potential list of anti-inflammatory agents.

REFERENCES

- Newal C.A., Anderson L.A., Phillipson J.D.: Herbal Medicines, pp. 1- 296, Pharmaceutical Press, London 1986.
- Newman D.J., Gragg G.M., Snader K.M.: Nat. Prod. Rep. 17, 215 (2000).
- Huffman M.A.: Natural Products Research Network for Eastern and Central Africa. Kenya 31, 13 (2001).
- Hensel H.: Pflugers Arch. 353, 191 (1975).
- T. Wang T., Taylor E.W., Andrade D., Abe A.S.: J. Exp. Biol. 204 3553 (2001).
- Miguel A.M., Alfredo D.C.: J. Biodiver. Conserv. 5, 957 (1999).
- Andrade D., Toledo L.F., Abe A.S., Wang T.: J. Exp. Biol. 207, 1379 (2004).
- Beckett A.H., Stanlake J.B.: Practical Pharmaceutical Chemistry. Part II, 3rd Ed. pp 148, 153, Athlone Press, London 1980.
- British Pharmacopoeia vol. 2, pp. A146,147, HMSO, London 1993.
- British Pharmacopoeia vol. 4, pp. A120, 121, HMSO, London 2003.
- le Grand P.A., Wondergem P.A., Verpoorte R., Pousset J.L.: J. Ethnopharm. 22, 25 (1988).
- Bailey R.W., Scott E.G.: Diagnostic Microbiology, 3rd Ed. pp. 136, 152, 204, The CV Mosby Co., Saint Louis 1970.
- Greenwood, D.: Antibiotic sensitivity testing In: Greenwood, D. (Ed.) Antimicrobial Chemotherapy. pp. 91, 100, Oxford University Press, New York 1989.
- Tubaro A., Dri P., Delbello G., Zilli C., Della L.R.: Agents Actions 17, 347 (1985).

Received: 30.01.2008