

# EFFECTS OF PETROLEUM ETHER, ETHANOL AND AQUEOUS EXTRACTS OF *INDIGOFERA CAPITATA* ON OEDEMA INDUCED ACTIVITY OF *NAJA NIGRICOLLIS* VENOM IN ENVENOMED RATS

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## ABSTRACT

The growing demand for herbal products has led to high increase of plant material traded across the world. *Indigofera* species provide some of these plants' materials. *Indigofera capitata* is a plant of the family Fabaceae. The acute toxicity and effects of pet.ether, ethanol and aqueous extracts of the plant were carried out in albino rats. The results of acute toxicity study revealed that pet.ether, ethanol and aqueous extracts had LD50 values of 774.6, 1131.4 and 3807.8mg/kg respectively. These helped to determine the safeness and dosage used for in vivo activity of each extract. The results of the effects of the extracts indicated all the plant extracts had significant ( $p < 0.05$ ) and dose-dependent Oedema forming activity against LD99 *Naja nigricollis* envenomed rats. Highest reduction in edema forming activity of the extract are shown at dose of 300mg/kg(ethanol) and 1000mg/kg (aqueous) with lowest edema forming activity of  $108.00 \pm 1.90$  and  $102.00 \pm 1.90$  (% mm) respectively compared with the positive control group. The finding revealed that the plant can be used for treatment of snake bite more especially *Naja nigricollis* bite.

**Keywords:** *Indigofera capitata*, Acute toxicity, Oedema induced activity, Pet. ether, Ethanol and Aqueous extracts

## INTRODUCTION

Medicinal plants are the local heritage with global importance. Various plants that were used against snake bite were reported to possess compounds with antsnake venom activities and most of these plants have no scientific validation (Antony et al., 2010). No much information documented on the uses of *Indigofera capitata* but the plant has been known as a potential source of traditional medicine against snake bite. The ethnomedical information of some *Indigofera* species shows that most of the species are highly valuable in traditional medicine. In Mexico the root of *I. suffruticosa* is used to overcome nervousness, against jaundice and lice. The dried leaf is used for treatment of ulcer and urinary diseases (Leite et al., 2004). In central Africa, the root of *I. sumplicifolia* is used to induce abortion when taken with hot water by pregnant women while the entire plant of *I. spicata* is used to cause abortion in pregnant grazing animal like cow, when administered orally and the dried root is used to treat schistosomiasis in human adult. The dried leaves of *I. arrecta* are used for treatment of diabetes, respiratory ailment, leprosy, malaria, scabies, sores, eczema and worm while the dried roots are used for stomach conditions; alleviate labour pain and tuberculosis (Saurabh et al., 2010).

The present studies aimed at investigating the effects of pet. ether, ethanol and aqueous extracts of whole plant on edema induced activity of *Naja nigricollis* envenomed rats with a view to either

support or debunk the traditional claims of using the plant in the management of snake bites.

## MATERIALS AND METHODS

### Plant Collection and Identification

The collection of *Indigofera capitata* and *I. conferta* was carried out in Kachia Local Government Area (Kachia Lat: 90 45' N, Lon:70 58' E, Al: 615m) Kaduna State. They were identified and authenticated by a taxonomist in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, and Zaria. Voucher specimen numbers of 1084 was assigned for them and herbarium specimens were deposited for future reference.

### Preparation of Plant Materials

The dried whole plant powdered materials of the two species were obtained by using mortar and pestle.

### Extraction of Plant Material

This was aimed at obtaining the various extracts of each of the two whole plant materials by successive solvents extraction method (Anas et al., 2010).

### Acute Toxicity Studies

The method described by Lorke (1983) was adopted. A pilot study was carried out with the extracts of the two plants in albino rats. Nine rats of three groups (three rats per each group) were used for pilot studies and each group was administered with 10mg, 100mg and 1000mg, respectively. From the result of the pilot studies, suitable dose intervals were chosen and used to conduct the final toxicity study. Four rats of four groups (one mouse per each group) were used for final toxicity of each extract.

### Oedema Forming Activity

The effect of the different extracts of the plant on Oedema forming activity of *Naja nigricollis* in Rats were carried out. The modified method of Diganta et al., (2013) was adopted. In this method, twenty-five (25) albino rats were used and equally divided into five groups accordingly. Group one (n= 5) received normal saline, group two (n=5) received LD99 of *Naja nigricollis* venom, group three (n=5) received doses of plant extracts and LD99 *Naja nigricollis* venom, group four (n=5) received doses of plant extracts and LD99 *Naja nigricollis* venom, group five(n=5) received doses of plant extracts and LD99 *Naja nigricollis* venom.

All albino rats treated with plant extracts were injected intraperitoneally (ip). Group two (2), the rats (n=5) were injected subcutaneously in the right hind foot pad with LD99 *N. nigricollis*

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while group three (3), four (4) and five (5) were treated with different doses of extracts of the plant (ip) after 1hr they were injected subcutaneously in the right hind foot pad with LD99 *N. nigricollis* venom while the left hind foot pad injected with normal saline served as control. Two hours (2 hrs.) later the thickness of the individual rat hind foot pads, (right and left) were measured with a small thread and transparent ruler calibrated in millimeter (mm). The increase in thickness due to Oedema was expressed as ratio of the thickness of oedematous right hind foot pad to the thickness of left hind foot pad (control) in percentage.

#### Data Analysis

Results obtained were statistically analyzed by using the Analysis of variance (ANOVA) and the results expressed as mean  $\pm$  SEM compared with the positive control group.

#### RESULTS

The results of different extracts (i.e. pet. ether, ethanol and aqueous) of *I. capitata* against LD99 were analyzed as shown in table 1-3. Table 1 showed the results of oedema forming activity of albino rats treated with different doses of petroleum ether extract against LD99 *N. nigricollis* venom, the effect of the extract in reducing the edema forming activity were very small, the extract has very little protection against the venom. The results of ethanol and aqueous extracts were found to be very effective against the venom more especially at higher doses of the two extracts and increases in doses of extracts also increase the protection or neutralization potential against the LD99 *N. nigricollis* venom as shown in table 2 and 3.

**Table 1:** Oedema forming activity in albino rats treated with different doses of petroleum ether whole plant extract of *Indigofera capitata* against LD99 *Naja nigricollis* venom.

Treatment groups	Oedema forming activity (mm)*
Group 1 Received no treatment	100.00 $\pm$ 1.90
Group 2 Received LD99 Venom	125.20 $\pm$ 2.40
Group 3 50mg/kg + LD99 venom	122.40 $\pm$ 1.90
Group 4 100mg/kg+LD99 Venom	122.20 $\pm$ 2.30
Group 5 200mg/kg+LD99 venom	121.00 $\pm$ 1.80

\*Results in mean  $\pm$  SEM, n=5; experimental groups are compared with control (group 2). Values obtained are statistically significant at P<0.05

**Table 2:** Oedema forming activity in albino rats treated with ethanol whole plant extract of *I. capitata* against LD99 *N. nigricollis* venom.

Treatment groups	Oedema forming activity (mm)*
Group 1 Received no treatment	100.00 $\pm$ 1.90
Group 2 Received LD99 Venom	125.20 $\pm$ 1.40
Group 3 75mg/kg + LD99 venom	120.20 $\pm$ 1.80
Group 4 150mg/kg+LD99 Venom	113.40 $\pm$ 1.90
Group 5 300mg/kg+LD99 venom	108.80 $\pm$ 1.90

\*Results in mean  $\pm$  SEM, n = 5, experimental groups are compared with control (group 2). Values obtained are statistically significant at P<0.05.

**Table 3:** Oedema forming activity in albino rats treated with aqueous whole plant extract of *Indigofera capitata* against LD99 *Naja Nigricollis* venom.

Treatment groups	Oedema forming activity (mm)*
Group 1 Received no treatment	100.00 $\pm$ 1.90
Group 2 Received LD99 Venom	125.20 $\pm$ 1.40
Group 3 250mg/kg + LD99 venom	112.80 $\pm$ 2.10
Group 4 500mg/kg+LD99 Venom	105.40 $\pm$ 1.90
Group 5 1000mg/kg+LD99 venom	102.00 $\pm$ 1.89

\*Results in mean  $\pm$  SEM, n = 5, experimental groups are compared with control (group 2). Values obtained are statistically significant at P<0.05.

## DISCUSSION

The results of Oedema forming activities in treated rats with the various extracts of *I. capitata* against LD99 *N. nigricollis* venom revealed that petroleum ether and ethanol extracts of the plant showed little significant effect in reducing the oedema induced activity of the venom but in the case of aqueous extract of the plant, it's showed significant ( $P < 0.05$ ) and dose dependent reduction Oedema induced activity of LD99 *N. nigricollis* venom. The significant effects in the oedema forming activity of *N. nigricollis* venom in treated rats. The neutralization potential of the various extracts was observed in ethanol and aqueous extracts of the two plant species which the effect increases with an increase in the doses of the extracts. The significant effect was found to be more effective at high dose 500mg/kg and 1000mg/kg of aqueous extracts of the two plants. The Oedema forming activity of *N. nigricollis* venom in positive control rats was formed as a result of the effect of the venom in the blood vessels which result into the swelling of right foot when compared with left hind foot that received normal saline. The swelling was due excess fluid trapped in the body's tissues that occurred as a result of accumulated tissues fluid in the surrounding tissues. This agreed with the finding of Abubakar et al., (2006), reported that *I. pulchra* showed inhibitory effect against the anticoagulant, hemolytic and PhospholipaseA2 activities of *N. nigricollis* venom by methanolic extract of the plant.

## Conclusion

The ethanol and the aqueous extracts of the two plants had significant ( $p < 0.05$ ) and dose-dependent oedema forming activity against LD99 *Naja nigricollis* envenomed rats. The extracts at doses of 300mg/kg(ethanol) and 1000mg/kg (aqueous) had the lowest Oedema forming activity of  $108.80 \pm 1.90$  and  $102.00 \pm 1.89$  (% mm) respectively compared with the positive control group, this signify that the plant may be used for treatment of snake bites as claims by some traditional healer.

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