Typhonium trilobatum (L.) Schott Shows Potency against Lymphatic Filariasis in Man

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ABSTRACT

Active fraction of the extract of mature, raw corm of *Typhonium trilobatum* (L.) Schott at 50- 250 and 10 µg/ml killed all the *Brugia malayi* larvae *in vitro* within 24 and 48h, respectively. Albino Wistar Rats (AWR), when administered with the extract by oral route, did not show any toxic symptoms or mortality; the LD_{50} value was >2000mg extract/kg body weight for acute and sub-chronic toxicity. Histopathological investigations did not show any damage to tissues of vital organs such as, liver, kidney, spleen, lung, heart, duodenum and brain in both male and female adult AWR. Clinical studies involving human volunteers suffering from lymphatic filariasis (LF) showed alleviation in sufferings from fever, pain, lymphadenitis with reduction in swellings of limbs. Case studies on 10 human volunteers in both sexes revealed that there was complete elimination of LF, showing progressive reduction of antigenemia to negative values through Enzyme-Linked Immuno-sorbent Assay ELISA) using Og4C3. The studies were approved by the Institutional Ethical Committee.

Keywords-*Typhonium trilobatum*, edible corm, *Wuchereria bancrofti*, *Brugia malayi* larvicidal activity, toxicity, histopathology, ELISA, Og4C3.

1. INTRODUCTION

Lymphatic Filariasis (LF), a neglected tropical disease caused by parasitic infection can result in an altered lymphatic system and abnormal enlargement of body parts, inflicting pain, severe disability and social stigma. Nearly 1.4 billion people in 73 countries worldwide are threatened by LF; over 120 million are currently infected and about 40 million are disfigured and incapacitated by the disease. Approximately, 80% of these people live in 10 countries i.e., Bangladesh, Democratic Republic of Congo, Ethiopia, India, Indonesia, Myanmar, Nigeria, Nepal, Philippines and the United Republic of Tanzania [1]. The economic effects of LF can be devastating, as

the people with disfigurement and disability due to lymphoedema, hydrocele and elephantiasis have reduced work efficiency and poor household income [2]. Worldwide, elephantiasis, lymphoedema, and genital pathology afflict 44 million men, women and children whereas, another 76 million have parasites in their blood and hidden internal damage to their lymphatic and renal systems [3]. India, Indonesia, Nigeria and Bangladesh together are reported to have about 70% of world's LF load [4]. LF is a major public health problem in India which costs estimated $1 billion annually to the national economy [5,6]. Although filariasis does not kill, it causes debility and imposes severe social and economic burden to the affected individuals, their families and the endemic communities. Lymphatic filariasis is the world's second leading cause of long-term disability. We still do not have any drug that destroy all the adult filarial worms in the lymphatics or reverse the pathology of this disease once it is established [7].

Corm extracts of *Typhonium trilobatum* (L) Schott, a herb (family Araceae), showed strong activity against LF [8,9,10,11]. Corm and tender leaf of *T. trilobatum* are cooked and eaten in the peninsular India, also used as poultice in the treatment of scirrhous and stomach complaints and applied in cases of venomous snake bites [12]. The raw corm of *T. trilobatum* is sometimes fed to domestic animals for de-worming in coastal Odisha [9].

2. MATERIALS & METHODS

2.1 Extraction of Active Compounds in *Typhonium trilobatum*

Mature and dormant corms of *T. trilobatum* were collected from the experimental garden of the Science Foundation for Tribal and Rural Resource Development (SFTRRD), Bhubaneswar, washed thoroughly under tap water for removal of root, soil etc., washed in distilled water, cut into 1 cm^3 and dried to constant weight in hot air oven at a temperature of 60^oC and stored for further use. The voucher specimen was deposited in the SFTRRD herbaria.

Extraction was made in rotary vacuum extractor (Rotavapor R-210/R-215, Buchi) at a temperature 40^oC under vacuum; based on preliminary studies, the active fraction of the extract was stored at -20^oC for use in subsequent experiments.

3. LARVICIDAL ACTIVITY

Active fraction of the extract dissolved in sterile distilled water at 10, 50, 100 and 250µg/ml were used in the toxicity studies. The larvae of *Brugia malayi* were separately introduced into these solutions in petri-dishes containing graded concentrations; in control no extract was dissolved in the distilled water, larval mortalities were recorded after 24 and 48 h of exposure following Abbott's correction [13].

4. TOXICITY STUDIES

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423 [14]. Separate experiments were conducted to study toxicity and LD_{50} value of the extract in the test animals. Histopathological studies were carried out to assess tissue damage of vital organs.

Healthy, adult, 6-8 week old Adult Wistar Rats (AWR) procured from a registered breeder were acclimatized to the laboratory conditions for a week prior to the test. Adult male and female rats (n=30) were divided into six experimental groups (5 rats/group) for the study (Table 1). While the animals (M/ F) in Group 1 and 2 served as control, they were given two oral doses of the extract at 200 mg/kg body weight to animals in Group 3 and 4 for sub-chronic and in Group 5 and 6, both M/F rats were administered with the extract at 400 mg/kg body weight for acute toxicity tests.

Table 1 Grouping of Male (M) and Female (F) AWR for sub-chronic and acute toxicity tests

Groups	No. of animals	Extract (mg/kg)	Duration of treatment (Days)	Frequency of oral treatment
1) Control (Female)	5	Distilled water	28	Daily
2) Control (Male)	5	Distilled water	28	Daily
3) Sub-chronic (Female)	5	200	28	Daily
4) Sub-chronic (Male)	5	200	28	Daily
5) Acute (Female)	5	400	01	Once
6) Acute (Male)	5	400	01	Once

The animals were maintained under controlled conditions at a temperature of 22±3^oC and relative humidity 55-60% RH with 12h light, 12h dark cycle throughout the experimental period. The animals were fasted over night prior to the administration of the extract. After treatment, food was withheld for 4h. Throughout the experimental period, standard laboratory diet and water were provided *ad libitum*. The test material (solid extract) was dissolved in water and administered in a single dose orally by gavage feeding.

The test animals were kept under observation for a period of 14 days after dosing. The feed, water intake and body weight were recorded daily during the observation period; mortality, other signs and symptoms,

were recorded twice on the day of dosing and once daily thereafter; at the end of the test period, the animals were weighed and sacrificed. All experimental protocols involved in this study were approved by Institutional Animal Ethics Committee.

Animals in groups 5 and 6 were sacrificed on day 14 and 1 and 4 were sacrificed on day 28. Blood samples were collected directly from the heart for haematology and clinical biochemistry on the day of sacrifice. Rats from different groups were anesthetized with sodium pentobarbital and perfused with 4% para-formaldehyde in PBS (pH 7.4). The vital organs such as brain, duodenum, liver, lungs, kidney, heart and spleen were removed, postfixed in 4% paraformaldehyde in PBS and further processed for histopathological analysis.

Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The stained sections were analyzed for microscopic evaluation, biochemical parameters of blood was recorded.

5. CLINICAL TRIALS

Clinical trials on 30 volunteers having swellings of different dimensions were carried out in two highly endemic coastal districts i.e. Kendrapara and Khurda of Odisha. The disease symptoms were recorded by specialist doctors with regard to the swellings, fever, pain and lymphadenitis. The swellings of the body were categorized into different grades i.e. Grade 1, Grade 2, Grade 3 and Grade 4 on the basis of severity of the disease; Grade-1 oedema reversible on elevation, Grade 2 oedema was partially reversible on elevation, Grade-3 oedema was irreversible with thickening of the skin and Grade-4 oedema was irreversible with papillary and nodular growth on the skin.

6. SEROLOGICAL TESTS

Highly sensitive and specific filarial antigen detection assay Og4C3 monoclonal antibody based ELISA test was carried out using serum samples to detect, quantify and monitor the antigenemia. The Og4C3 circulating antigen detection test with 10 volunteers was done by ELISA according to the manufacturer's recommendations (TropBio Pty Ltd, Townsville, Australia). Sera from healthy people, having negative A.U. value based on ELISA, Og4C3 reports, were used as controls. These tests were done before the administration of the active fraction of the extract and subsequently at 90 day intervals after administration of the extract to diagnose, quantify and monitor the infection in course of the investigation. The data were collected and analyzed using Excel 6.0 software.

7. CASE STUDIES

Volunteers were kept under constant observation, administered with the extract twice daily followed by periodic measurements of the swollen limbs, intensity of the fever, pain and lymphadenitis. Quantification and monitoring of antigen unit was done by ELISA Og4C3 antigen detection assay every 90 day intervals after intake of the extract.

8. RESULTS

8.1 Larvicidal activity

In vitro studies with the active fraction of the *Typhonium trilobatum* extract showed strong activity against *Brugia malayi* killing all the larvae at 50, 100 and 250 µg/ml in 24h; at 10µg/ml, all the larvae were observed dead within 48 h (Table 2).

Table 2 *In vitro* larvicidal (*Brugia malayi)* activity of the extract in 24 and 48h

Sl. No	Conc. (µg/ml)	Live						Dead						Recovery		Mortality (%)	
		24h			48h			24h			48h			24h	48h	24h	48h
1	Control (0)	124	126	125	118	121	119	3	2	3	8	7	8	128	128	3.0	7.5
2	10	55	77	66	0	0	0	63	35	49	125	126	125	125	125	41.2	100
3	50	0	0	0	0	0	0	125	134	129	125	134	129	129	129	100	100
4	100	0	0	0	0	0	0	121	121	121	121	123	122	122	122	100	100
5	250	0	0	0	0	0	0	123	123	123	123	129	126	126	126	100	100

8.2 Acute oral toxicity

During the dosage regimen, no abnormal behaviour with regard to the food and water intake was observed. Hematoxylin & Eosin staining of paraffin-embedded 5 micron-thick sections of the liver, kidney, spleen, lung, heart, duodenum and brain at magnification 200× and 400× are presented in Figure 1.

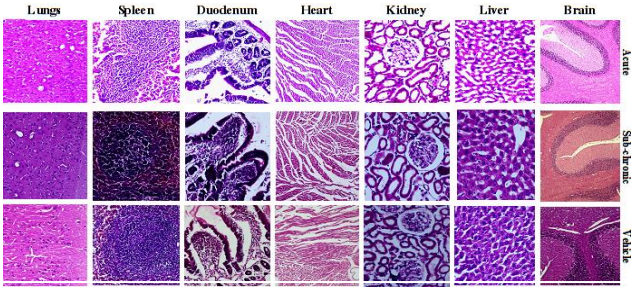

Figure 1 H&E staining of paraffin-embedded 5 micron-thick sections of the liver, kidney, spleen, lung, heart, duodenum and brain at magnification 200x and 400x.

The liver showed normal hepatic lobular architecture; the kidneys revealed normal glomeruli, proximal and distal tubules, interstitium, and blood vessels; the splenic follicles and vascular sinusoids were indistinguishable between the treated and control groups; the lung tissue showed normal alveoli and the heart muscle showed normal morphology among the two groups. Microsections of brain did not reveal any infracted areas; the cerebral cortex, gray and white matters appeared normal. The gut showed normal mucosa, submucosa and muscularis mucosa. There was no significant difference in haematological and blood biochemical parameters between the treated and untreated groups (Figures 2 a-e).

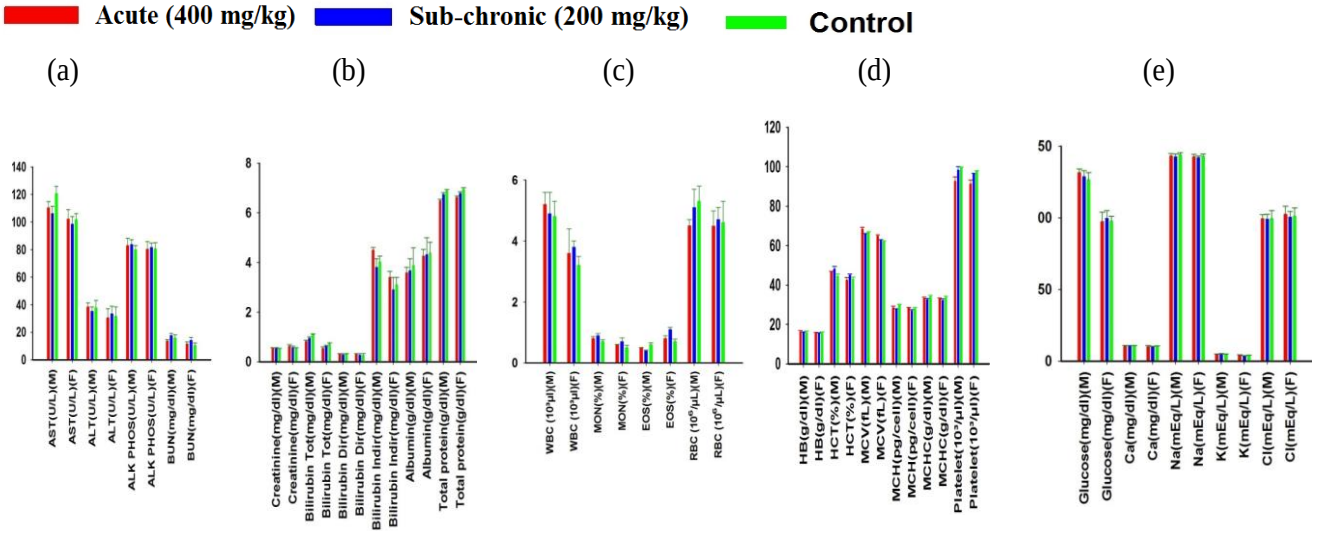

Figure 2 (a-e) Organ functions (a) and (b) show undistinguishable profiles among the three groups, Acute (400 mg/kg), Sub-chronic (200 mg/kg) and Control for both male (M) and female (F) groups. No significant differences could be detected in the WBC count (WBC), monocytes (MON), eosinophils (EOS), RBC count (RBC), haemoglobin concentration (HB), Haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count between treated and untreated groups (c) and (d). Also there was no much difference in Serum Glucose, Serum Ca, Na, K and Cl levels between the treated and untreated groups (e).

8.3 Pharmacological observations

No toxic signs, symptoms and mortality were noted in the treated Wistar Abino Rats (WAR) at any of the doses including the highest dose at 2000mg extract/kg body weight. The studies showed that the active fraction of the extract was non toxic at 2000mg/ kg of body weight; LD50 value was > 2000mg extract/kg body weight.

8.4 Clinical trials

Twenty one out of 30 volunteers i.e. 70% cases showed reduced swelling after 15 days of administration of the extract and 25 of 30 (83%) showed reduction in their swellings after 30 days of treatment. Amongst 30 volunteers, 23 i.e. 77% cases had fever before intake of the extract; twenty out of 23 volunteers (87%) were relieved from fever after 15 days of administration of the extract and after 30 days all of them i.e. 100% of the volunteers were relieved of fever. Twenty one volunteers had pain before treatment out of which 18 i.e. 86% were relieved from pain after 15 days of administration of the extract and after 30 days all the volunteers were relieved from pain. Out of 30 volunteers, only 8 had lymphadenitis which got subsided after 15 days of treatment. Out of 30 cases studied, 1 volunteer had Grade 1 oedema reversible on elevation, 3 had Grade 2 oedema of the limb which was partially reversible on elevation, 25 had Grade 3, irreversible oedema of the limb with thickening of the skin and 1 belonged to Grade 4 oedema, irreversible oedema of the limb with papillary and nodular growth. All the volunteers were able to attend to their normal routine, day to day activity and the disability could be overcome after 30 days of administration of the extract.

8.5 Case Studies

Detection, quantification and monitoring of the antigenemia among the volunteers during the period of study were done using ELISA for the *Wuchereria bancrofti* specific Og4C3 antigen at 90 day intervals. Intake of the extract caused progressive reduction to negative antigen (Table 3).

Table 3 Decrease in Antigen Units (A.U.) by treatment with *Typhonium trilobatum* extract as revealed by serological (ELISA, Og4C3, Filaria) tests

Sl. No	Volunteers	Sex	1st Testing		2nd Testing		3rd Testing		4th Testing		Remarks
			Date	Antigen Units (A.U.)	Date	Antigen Units (A.U.)	Date	Antigen Units (A.U.)	Date	Antigen Units (A.U.)	
1	A	M	15.01.10	512	15.04.10	256	15.07.10	128	15.10.10	128	Negative
2	B	F	15.01.10	512	15.04.10	256	15.07.10	128	15.10.10	128	Negative
3	C	M	15.01.10	256	15.04.10	128	15.07.10	128	15.10.10	128	Negative
4	D	F	15.01.10	256	15.04.10	256	15.07.10	512	15.10.10	128	Negative
5	E	M	15.01.10	512	15.04.10	128	15.07.10	128	15.10.10	128	Negative
6	F	M	15.01.10	512	15.04.10	128	15.07.10	128	15.10.10	128	Negative
7	G	F	15.01.10	2048	15.04.10	512	15.07.10	256	15.10.10	128	Negative
8	H	F	15.01.10	512	15.04.10	256	15.07.10	256	15.10.10	128	Negative
9	I	F	15.01.10	256	15.04.10	128	15.07.10	128	15.10.10	128	Negative
10	J	M	15.01.10	512	15.04.10	256	15.07.10	256	15.10.10	128	Negative

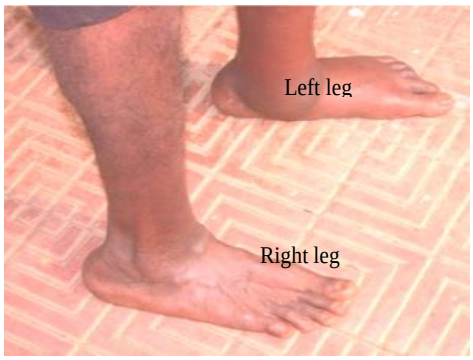

Figure 3a Swelling of leg before use of extract,

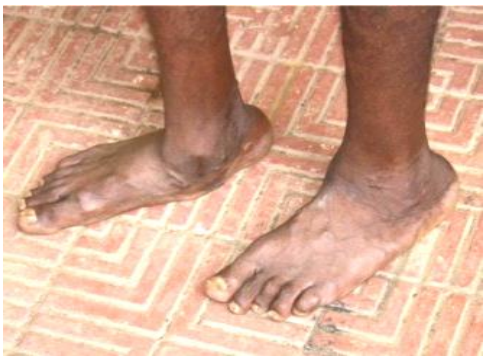

Figure 3b Reversal of swelling to the normal state

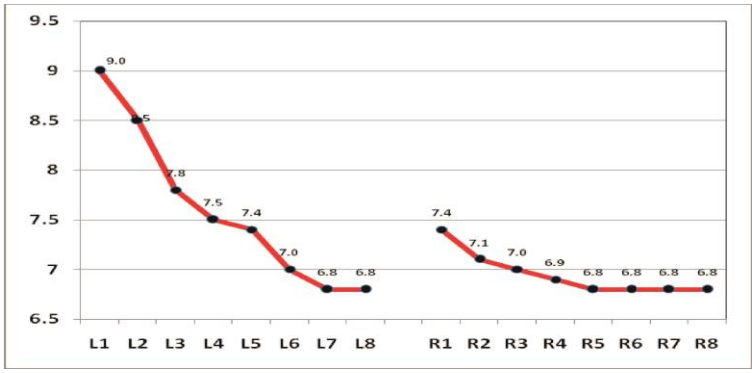

Figure 4 Gradual decrease in swelling of left & right leg on administration with the extract

LF was cured in 10 volunteers as is evident from antigen detection assay (ELISA, Og4C3) and there was no recurrence of fever, pain, lymphadenitis in any of the volunteers; Grade 1 cases reversed back to the original state; Grade 2, Grade 3 & Grade 4 cases showed significant and progressive reduction in swelling without any sign of toxic symptoms.

There was complete cure of Filariasis within 6-12 months of intake of the extract showing decrease in Antigen Units (A.U.) to negative values (A.U. 128) as revealed by Serological Tests (ELISA, Og4C3, Filaria, Figures 3 a, 3 b and Figure 4).

Ethanol:water (1:1) fraction of *Typhonium trilobatum* extract showed strong larvicidal activity against *Brugia malayi*. The active fraction of the extract was safe, not toxic nor caused any damage to the tissues of the vital organs such as liver, kidney, spleen, lung, heart, duodenum and brain in both male & female adult Albino Wistar Rats (AWR). Negative A.U. (128 or less) values in serological (ELISA, Og4C3) tests indicated death of adult worms (*Wuchereria bancrofti)* and elimination of Lymphatic Filariasis (LF) in man.

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REFERENCES

[1] WHO (2014), World Health Organization Fact Sheet No. 102.

[2] Zeldenryk, L. M., Gray, M., Speare, R., Gordon, S., and Melrose, W. (2011), "The Emerging Story of Disability Associated with Lymphatic Filariasis: A Critical Review." PLoS Neglected Tropical Diseases. 5(12).

[3] Melrose W S, (2011), The Emerging Story of Disability Associated with Lymphatic Filariasis: A Critical Review. PLoS Negl Trop Dis 5(12). e1366 doi:10.137

[4] Ottesen EA, Duke BO, Karam M, Behbehani K (1997). Strategies and tools for the control/ elimination of lymphatic filariasis. Bulletin of the World Health Organization 75, 491- 503.

[5] Hotez PJ, Fenwick A, Savioli L, Molyneux DH (2009), Rescuing the "bottom billion" through neglected tropical disease control. Lancet.; 373:1570–1574.

[6] Ramaiah KD, Das PK, Michael E, Guyatt H (2000), The economic burden of lymphatic filariasis in India. Parasitol Today 16(6):251-3.

[7] Shenoy, RK (2012), In Perspectives of Indian Medicinal Plants in the management of Lymphatic Filariasis, ICMR.

[8] Das P and Das AB (1998), Esterase marker during callus mediated regeneration of *Typhonium trilobatum* Schott, A medicinal Plant. The Orissa J. of Horticulture. Vol 26 (2). 50-55.

[9] Das P, Palai SK, Patra A, Samantaray S, Rout GR (1999), *In vitro* somatic embryogenesis in *T. trilobatum* Schott. Plant Growth Regulation 27: 193-197.

[10] Das P (2003), In vitro regeneration of *Typhonium trilobatum* Schott: A potential antifilarial plant via somatic embryogenesis. III World Congress on Medicinal and Aromatic Plants for Human Welfare. Chiang Mai, Thailand, February 3-7, 2003.

[11] Das P, Dey S, Das AB, Mohanty S, Rao HBDP (2006), Variation in chromosome number, karyotype and nuclear DNA content of five species of Indian Typhonium Schott (Araceae)- A Medicinally Important Plant. Cytologia 71(4): 371-377.

[12] Anonymous, (1974), The Wealth of India, CSIR, India.

[13] Abbott WS (1925), A method of computing the effectiveness of an insecticide. J Econ Entomol; 18 : 265-7.

[14] OECD (2011), OECD Guidelines for the Testing of Chemicals (No. 423) "Acute Oral Toxicity-Acute Toxic Class Method" (Adopted on 17 December 2011).