ISSN 2278 – 540X FISSN 2278 – 5396

DRUG DISCOVERY

In-vivo actions of Methanolic extract of *Lantana camara* on the total protein and free aminoacids content in *Culex* mosquito larvae

Divya S Rajan

Department of Zoology, S.N.College, Kollam, Kerala-691001, E.mail: divyashyju2010@gmail.com

Received 29 July; accepted 17 September; published online 01 October; printed 16 October 2013

ABSTRACT

In the present study as the methanolic extract of *Lantana camara* showed significant toxicity, that extract was selected for the study of invivo action of *Lantana camara* extract on the total protein and free aminoacid content in the *Culex* mosquito larvae. In the laboratory condition, 85mg/100ml was found to be very effective in creating 100% mortality of the mosquito larvae. Fourth in star mosquito larvae was put in a lethal concentration of the extract and kept them for time periods such as 2,4 and 6 hours. The LD_{50} of methanolic extract of *Lantana camara*, with respect to the larvae and was almost found to be 40mg/100ml. Within this period, the larvae showed visible symptoms of toxicity such as lack of co-ordination of movements, incapability of surfacing to take oxygen and poor response to stimuli such as touching with glass rods. A very simple observation on the pH of the larval homogenate of control and drug treated larvae showed that the homogenate of normal larvae was alkaline (pH 7.1 to 7.2) but in treated and intoxicated larvae the pH of homogenate was slightly acidic (pH 6.8 to 6.9). The protein content of whole body on wet weight basis was more than 3%.On incubating mosquito larvae in lethal concentration of *Lantana camara* extract, there was a gradual increase of total free amino acid together with gradual decrease in protein content. The study indicated that at the sixth hour of incubation almost 1/3rd of protein was depleted and there was a 50% increase of aminoacid that together resulted in the death of the larvae. Thus the findings proved to suggest the use of the above information in future for the formulation of effective antilarvicidal agent from the invasive weed *Lantana camara*, that prevents the spreading of dreadful mosquito borne diseases.

Key words: In-vivo analysis; proteolytic activity; *Lantana camara;* free aminoacid; methanolic extract

To Cite This Article
Divya S Rajan. *In-vivo* actions of Methanolic extract of *Lantana camara* on the total protein and free aminoacids content in *Culex* mosquito larvae. Drug Discovery, 2013, 6(16), 10-12

1. INTRODUCTION

The mosquito is the principal vector of many vector borne diseases affecting human beings and other animals. Of all the existing insect pests, mosquitos are a great nuisance due to the infection of very painful bites, the mere whine of their wings is enough to disturb us from our sleep. Many works have been conducted, especially in India, on the chemical constituents of *Lantana* extracts from the leaves exhibit antimicrobial, fungicidal, insecticidal and nematicidal activity (Chavan & Nikam, 1982; Dua et al; 1986; Sharma and Sharma 1989, Begum et al. 2003). Insects are characterized by having high concentration of free aminoacids which was once proposed to be a taxonomic character of that class. In the haemolymph, the total aminoacid load amounts 100 to 500 times higher than that in human blood (Evans & Kaleysa Raj, 1992). Exposure to certain insecticides drastically affects the equilibrium of aminoacid pool, causing significant reduction and increase in aminoacids (Evans and Kaleysa Raj, 1992).

Figure 1
Culex mosquito

Testing of larvicidal activity in various organic solvents revealed that the methanolic extract showed maximum larvicidal efficacy against the mosquito larvae. The findings of the present study revealed on incubating larvae in lethal concentration of *Lantana camara* extract, there was a gradual increase of total free aminoacid together with a gradual decrease in protein content. *Lantana camara* due to its anti fungal and antibacterial activity as a larvicide it never alters the equilibrium of ecosystem and human life since it is non toxic to human.

2. MATERIALS AND METHODS

The plant *Lantana camara* was collected locally from the remote areas of Alappuzha district of Kerala that forms one of the main centres for the incidence of mosquito borne diseases. The leaves were put in shade and for five days. Two gram of plant powder was weighed and put in 250 ml conical flask and 200ml distilled water was poured into it. The mixture after 24 hours extraction was filtered with the help of coarse filter paper. The plant powder was discarded and the extract was dried by keeping it in hot air oven at 45 ^{0}C for recording the yield. Organic solvents such as methanol and chloroform were also used for extraction following the same procedure. The extract was made free of organic solvents by keeping known volume of extract under fan. The dry extract got through the extraction with solvents was kept in dessicator containing calcium chloride. Different concentration of the extract was made by diluting with water and each concentration was separately tested and percentage of mortality after six hours for each concentration was recorded.LD_{50} was determined from a

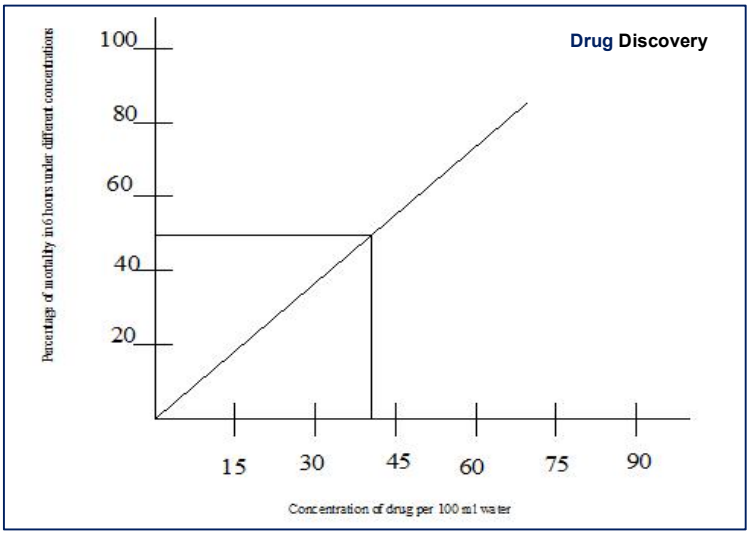
Table 1
Extraction from the dry powder of *Lantana camara* by different solvents

Solvents used(200 ml)	Nature of extract	Plant sample used(gm)	Weight of drug extract(mg)	Percentage of yield	Minimum quantity required in 100 ml
Water	Aqueous extract	2	200±12	10	110 mg
Methanol	Methanolic extract	2	160±10	8	85mg
Chloroform	Chloroform extract	2	100±5	5	90mg

Table 2
Invivo action of *Lantana camara* extract on total protein, free aminoacidsin *Culex* mosquito

Biochemical parameters	Normal control	Incubation in drug (methanolic extract of *Lantana camara)*		
		2hr	4hr	6hr
Total protein(**)	3.26±0.2	3.1±0.2	2.8±0.12	2.01±0.1
Total free aminoacid(*)	460±26	510±22	580±29	621±3

*Values are expressed in micrograms free amino acid/100mg wet tissue.
**Values are expressed in percentage of protein/100 mg wet tissue.



Graph 1
Determination of LD $_{50}$ of methanolic extract of *Lantana camara* on *Aedes aegypti* larvae

graph by plotting % of mortality against concentration. Mosquito larvae reared in the laboratory necessitated 85mg/100 ml concentration of methanolic extract for 100% mortality in six hours. The LD_{50} of methanolic extract of *Lantana camara*, with respect to the larvae and as per the figure is almost 40mg/100ml.

Fifty larvae were put in lethal concentration of plant extract and incubated for 4 hours. After incubation period, they were separated from the medium, washed in distilled water and blotted in filter paper. The larvae were homogenized in distilled water (5ml) and kept in ice cold condition and another set of larvae not kept in plant extract was used as control sample. Both samples were brought to normal temperature and pH of the homogenate was tested with the help of pH meter. Fourth in star larvae of *Culex sp* in a lethal concentration of test compound and kept for different periods such as 2, 4 and 6 hours. At various appropriate periods of incubation they were separated from the drug containing medium, washed in ice cold water and homogenized in ice cold condition phosphate buffer system and that homogenate was used protein and free aminoacids (Lowry *et al;*1954).Appropriate controls were followed for comparison. Estimation of protein was done by using Folin's reagent (Lowry et al. 1951). Total free aminoacids was estimated using perchloric acid leucine standard (Spies, 1957).

3. RESULTS AND DISCUSSION

A very simple observation on the pH of the larval homogenate of control and drug treated larvae was alkaline (7.2 to 7.4) but in treated and intoxicated larvae the pH of the homogenate was slightly acidic (pH6.7 to 6.8).The change of pH from alkaline to acidic will be sufficient for inhibiting or blocking most of the enzymatic reactions leading to the death of organisms. This is in conformation with the findings of Mittal (2003). The LD_{50} of methanolic extract, with respect to the larvae and as is almost 40mg/100ml (Graph 1). The larvae necessitated 85mg/100ml concentration of the methanolic extract for 100% mortality in 6 hours (Table1).

The mosquito larva (fourth stage) has a very high concentration of total free aminoacids when tested with the whole body homogenate (Table 1). The protein content of whole body on wet weight basis was more than 3%.On incubating mosquito larvae in lethal concentration of *Lantana* extract, there was a gradual increase of total free aminoacids together with gradual decrease in protein content. In mosquito larvae, intoxication by the plant extract resulted a gradual increase of free aminoacids (Table 2). At the sixth hour of incubation, they were almost died and at that time there was a 50% increase of free aminoacids. Increase of free aminoacids was coupled with depletion of protein content. At the sixth hour of incubation, almost 1/3rd of protein was depleted in larvae.

The observed results indicated that there was elevation of proteolytic activity resulting in the depletion of protein content of the body with the enhancement of free aminoacid content. It was observed that when mosquito larvae were kept in spring water containing the extract, paralysis of larvae occurred. So the invivo action was conducted with an assumption that aminoacid metabolism and neurotransmission may be affected by *Lantana camara* extract. Glutamic acid and in some instances asparatic acid appear to be very important transmitter candidate in the somatic neuromuscular transmission in insects and are excitatory in function. In the insect nervous tissue glutamate can be produced from glutamine by the action of glutaminase from the tricarboxylic acid intermediate, α-ketoglutarate by the action of transaminase or glutamate dehydrogenase or possibly from ornithine or proline. Toxins can drastically affect the metabolism of glutamate (Evans &Subramoniam, 1992).

When mosquito larvae were kept in spring water containing *Lantana camara* extract,paralysis was observed.So mode of action study was conducted with an assumption that aminoacid metabolism and neurotransmission may be affected by *Lantana camara*.The observed paralysis in the intoxicated larvae may be due to accumulation of glutamic acid in the body.A paralysed larvae is unable to surface the water for getting oxygen. This might have indirectly contributed to the death of the larvae.

Extract from the leaves of *Lantana* possessed larvicidal activity while extract from flowers of the plant showed repellant activity against mosquitos (Dua et al 1996; 2003; 2010).Thus the present investigation represents *Lantana camara* as a valuable plant and can be utilized as an effective candidate for future drug development that can bring about a reduction in the amount of incidence of

Divya S Rajan,
In-vivo actions of Methanolic extract of *Lantana camara* on the total protein and free aminoacids content in *Culex* mosquito larvae,
Drug discovery, 2013, 6(16), 10-12,
http://www.discovery.org.in/dd.htm

mosquito borne diseases through the biological control of vectors.

REFERENCES

1. Begum S, Wahab A, Siddiqui BS. Pentacyclic triterpenoids from the aerial parts of *Lantana camara*. *Chem Pharm Bull. 2003,* 51, 134–137
2. Chavan, SR, Nikam ST. Investigation of *Lantana camara Linn*(Verbenaceae) leaves for larvicidal activity. *Bull.Haff.Inst;* 1982, 10, p21-22
3. Dua VK, Gupta NC, Pandey AC, Sharma VP. REpellancy of Lantana camara (Verbenaceae) flowers against *Aedes* mosquito. *J.Am.Mosq.Control.Assoc.* 1996, 12, 406-408
4. Dua VK, Pandey AC, Dash AP. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian Journal of Medical Research.* 2010, 131, 434-439
5. Dua VK, Pandey AC, Singh R, Sharma VP, Subbarao SK. Isolation of repellant ingredients from Lantana camara (Verbenaceae) flowers and their repellency against *Aedes* mosquitos. *J. Appl.Entmol* 2003, 127, 509-511
6. Evans DA, Kaleysaraj R. Larvicidal efficacy of Quassia against Culex quinquefasciatus. *Indian.J.Comp.Anim.physiol.*1992, 1, 46-54
7. Evans DA, Subramoniam A. Influence of plant extracts on immune system of mice. *Indian J.Med,Res.* 1992, 18, 33-38
8. Ghisalberti EL. *Lantana camara* Linn. (Review). *Fitoterapia*. 2000, 71, 467–485
9. Lowry OH. Rosenbrough NJ, Fare AL, Randall RJ. Protein measurement with the Folin,s Reagent. *J.Biol.Chem*. 1951, 193, 265-275
10. Mittal PK. Bio-larvicides in vector control challenges prospects. *J.Vect.Borne.Dis.* 2003, 30-32
11. Sanjeeb Kalita, Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao. A review on medicinal properties of *Lantana camara* Linn. *Research.J.Pharm and Tech* 2012, 5(6), 711-717
12. Sharma OP, Dawra RK, Makkar HPS. Toxicity of isolated Lantana (*Lantana camara Linn.*) constituents to male and female guinea pigs. *Vet Hum Toxicol*. 1989, 11, 10–13
13. Spies JR. Colorimetric procedures for amino acids, *In: Methods in Enzymology .VolII. Academic press, New York*. 1957, 461-471

Divya S Rajan,
In-vivo actions of Methanolic extract of *Lantana camara* on the total protein and free aminoacids content in *Culex* mosquito larvae,
Drug discovery, 2013, 6(16), 10-12,
http://www.discovery.org.in/dd.htm