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A study on phytochemical & anti-nociceptive activity of *lonicera ligustrina* leaf extract

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ABSTRACT

In this study we aimed to elucidate over pharmacological activities of Lonicera-ligustrina (Caprifoliaceae).The ethanolic extract of L. ligustrina (LL) dose dependently had shown analgesic activity. The anti-nociceptive activity of LL was assessed using the Tail-flick model & Tail-immersion model in swiss albino mice. EELL showed the presence of Flavonoids, Terpenoids, Steroids, Alkaloids, Glycosides etc& showed significant analgesic effect with significant increase in reaction time against thermal stimuli & it attributed through decrease in pain due to biogenic amines BK,5 HT, PG's, NA, NGF, Neuropeptides (SP,CGRP, Interferons&Enkephalins).

Keywords: Nociception, Maximum tolerated Dose, Libitum

INTRODUCTION

Medicinal plant, an important element of medical systems in all over the world. For natural drug research and development, ethno botany provides a rich resource . Traditional medicine,the most and easily accessible & affordable source of treatment in the health care system of poor communities. Usage of traditional plants for medicinal purposes have been a long history for local people & natural herbal preparations as medicine was first used by Chinese. According to writings, the usage of plants for medicinal purpose is as old as 4000-5000 B.C.

The genus Lonicera (Honey suckles) belongs to the family Caprifoliaceae and comprises of about 12 genera and 450 species, occurs mainly in Northern Hemisphere temperate regions. In Pakistan, 4 genera of Lonicera and 27 species are represented. Various species except ligustrina of this genus are used in the treatment of acute fever, respiratory infections, head ache, , antibacterial, antioxidant, cytoprotective, hepatoprotective, antiviral, antitumor and anti-inflammatory activities[1]. In this investigation, ethanolic extract of Loniceraligustrina fraction was obtained and the anti-nociceptive activity was evaluated using several experimental animal models of nociception. In Nociception intense thermal stimulation, chemical or mechanical stimulation of sensory nerve cells called nociceptive receptors produces signal ehich travels along the chain of nerve fibers via the spinal cord to the brain. Loniceraligustrina Wall belonging the family of Caprifoliaceae, Shrubs, evergreen, semi evergreen, or deciduous, 1.5–2.5(–5) mts taller. Winter buds which are with

several pairs of acute scales. Young branches are with stiff and upwardly curved hairs. Leaves are ovate to lanceolate , 0.4–8 × 0.2–1.5 cm, papery or leathery, abaxially arranged sometimes with minute black glands, abaxially usually glabrous, shiny or with sparse short hairs and reddish glandular hairs, midvein slightly impressed, flat or raised, stiff hair, base[1] .The present study deals with the ethanolic extract of LL & screening of phytochemicals present in LL & to study the Anti-nociceptive activity on swiss albino mice.

MATERIALS & METHODS

Diclofenac sodium;Ethanol;Tween-80;Distilled water;Reagents

The fresh leaves of Loniceraligustrina were collected and authenticated by botanist Dr. Madhavachetty, Assistant professor,Department of Botany in S.V. University, Tirupathi, and voucher specimen number (1189).

Fresh leaves of Loniceraligustrina were cleaned and dried under shade in clean dust free environment, grinded and stored in an air-tight container. The total 50 g of coarse powder of LL was used & extracted with 250ml of 90% ethanol in a soxhlet apparatus[7] at 60- 75°C for 48 hrs. The extract was concentrated by evaporation. The yield was about 5.0% and stored at 4˚C for future use. The above extracted yield was taken to perform Phytochemical screening & the results are tabulated below in Table 2.

Table1: Preliminary-phytochemical screening

Chemical Category	Name of test
Carbohydrates	Molishs test
	Bial's test
Proteins & Amino acids	Biuret test
	Xanthoprotein test
	Millon's reagent test
Alkaloids	Mayer's test
	Hager's test
	Wagners Test
	Tannic Acid
Glycosides	General Test
	Borntragers test.
	Cardiac Glycosides
	Coumarin Glycosides
Phenolics / Tannins	Ferric chloride test
	Drug + lead acetate + water
	Potassium dichromate
Flavonoids	Shinoda's Test
	NaOH test
Saponins	Drug + water + shaking
Fixed oils & Fats	Spot test
Steroids	Libermann-Burchard test

Table2: Preliminary-Phytochemical screening[8-10].

S.NO	TEST	INFERENCE
1	Alkaloid	+
2	Carbohydrates	+
3	Flavonoids	+
4	Glycoides	+
5	Saponins	+
6	Steroids	+
7	Terpenoids	+
8	Tannins	+

The solid EELL was dissolved by using 1 % v/v Tween-80 as a vehicle for i.padministration [2-4].

Swiss albino mice (15-20)g, was maintained under the standard conditions of (27 + 20C; relative humidity 60 + 5%, light dark cycle for 12 hrs) and standard pellet diet feed and water adlibidum were used for present study[5] . All the experimental protocols were duly approved by Institutional Animal Ethics Committee (Reg. No: 1477/Re/s/11/CPCSEA), Dhanvanthri College of Pharmaceutical sciences, Mahaboobnagar, Telangana, India.

Determination of maximum tolerated dose [MTD]

Swiss albino mices are taken under the study of limit test. Three animals were kept for overnight fasting prior to drug administration and treated with 2000mg/kg as a single dose of Loniceraligustrina leaf extract. Via i.p. route as per the limit test of OECD guide lines 423 and the food was with held for further 3-4 hours and the mices were individually observed individually atleast once prior to 30minutes after dosing & periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for the total of 14days, for neurological, behavioral, and autonomic profiles (skin and fur, eye and mucous membranes, and also, circulatory, respiratory autonomic and central nervous systems, and somatomotor activity and behavioral pattern.

Attention should be directed to observe the salivation, convulsions, tremors, lethargy, sleep,coma& diarrhea) and for any lethality, morbidity and mortality. As per animal body weight required amount of standard drug (25 mg/kg) of Diclofenac sodium taken and dissolved in distilled water Loniceraligustrina leaf extract was used for trituration with 1ml of Tween 80 and 9ml of water added to form a suspension and dose was calculated as per animal body weight.

Experimental design

Swiss albino mice weighing around 150-200g. were divided in five groups, each group consisting of about six animals is treated as described below in table 3

Table 3:

Group No	Group Name	No. of Animals	Treatment
I	Control	6	Solvent 10ml/kg,b.wt (i.p.)
II	Standard	6	Diclofenac sodium 25mg/kg, b.wt (i.p)
		Tail immersion method	
III	TEST I	6	150mg/kg extract (i.p)
IV	TEST II	6	300mg/kg extract (i.p)
V	TEST III	6	150mg/kg extract (i.p)
VI	TEST IV	6	300mg/kg extract (i.p)

TAIL IMMERSION METHOD

This test was performed by using a tail immersion method, where swiss albino mice of either sex were divided in to four groups, each containing six animals. The basal reaction timeof all animals were recorded. A water bath was maintained at 510C (+ 0.5) using thermostatic setting provided which is provided with an instrument.

A thick paper rolled in to make a cone with a hole in the bottom & each animal were taken in cone and allowed to extend tail out through bottom hole. 2cm length of a tail was marked using marker pen and was dipped to note the response. Tail flick out by the mice of the hot water and body jerks were taken as response by using stop watch starting from the time of dipping to the time of flick, responses were recorded. A cut off period of 60sec was observed to avoid damage to tail.

The latency period was recorded at 30 min interval up to 90 min for control, standard and test drug administration.

RADIANT HEAT/TAIL FLICK METHOD

The tail flick test was used to calculate an analgesic activity by following the method defined by D'amour and Smith 1941 [6], with minor alterations in a given procedure. This tail flick method was utilized to study analgesic activity in mice.

A radiant heat automatic tail flick analgesiometer was applied to measure reaction latencies. Basal reaction time was recorded for the radiant heat by observing the tip (last 1-2 cm) of the tail on radiant heat source. The tail removal from the radiant warmth was taken as end point. To avoid injury of tail by radiant heat ,cut off time of 15 seconds was used. Miceswere divided into five groups, each contains six mice. Treated with standard (25 mg/kg), control, and extract (150, and 300 mg/kg)was followed.Tail-flick response with the latent period was determined at 30min, 45min, 60min, 75min, and 90 minutes after drugs administration.

Table 2: Data of EELL (Ethanolic extract of LoniceraLigustrina) by Tail Immersion Method

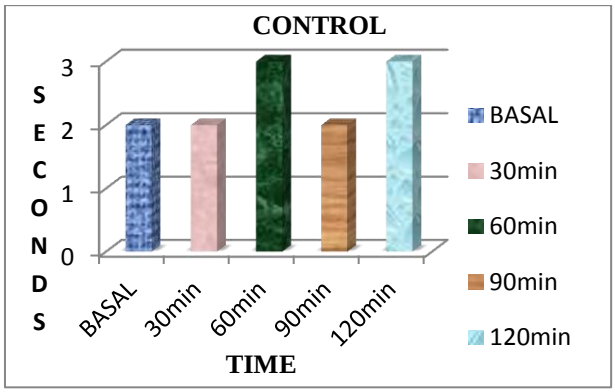
Group	Treatment	Dose (per kg/i.p.)	Swiss albino mice	Reaction time(sec) Post-Administration				
				Basal	30min	60min	90min	120min
Control	Distilled water	10ml	1	2	2	2	3	4
			2	3	2	3	2	3
			3	2	2	2	1	2
			4	3	2	4	3	2
			5	4	2	3	4	3
			6	3	3	4	3	4
	Diclofenac sodium	25mg/kg	1	2	2	2	3	9
			2	3	3	2	6	8
			3	2	4	4	3	2
Standard								
			4	2	2	4	6	8
			5	3	2	2	4	8
			6	3	3	2	4	3
Test-I	EELL	150mg/kg	1	2	3	3	4	6
			2	3	4	3	4	8
			3	2	3	6	8	10
			4	1	2	4	9	10
			5	2	3	5	6	8
			6	2	4	9	8	10
Test-II	EELL	300mg/kg	1	2	4	3	5	8
			2	3	3	2	4	9
			3	3	3	4	6	8
			4	2	3	6	9	10
			5	4	3	6	8	9
			6	3	5	7	8	10

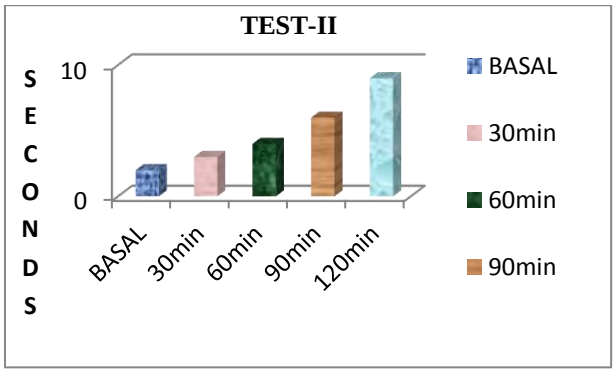
Table 3: Mean data of Analgesic activity of EELL by Tail immersion method on Swiss Albino Mice.

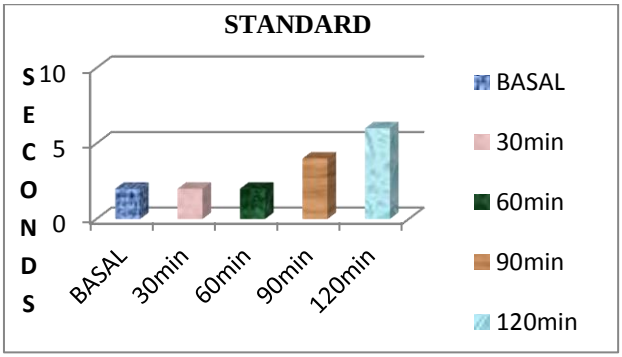
Group	Treatment	Dose (per kg/i.p.)	Reaction time(sec) Post-Administration				
			Basal	30min	60min	90min	120min
Control	Distilled water	10ml	2.83+0.31	2.50+0.37	3+0.37	2.67+0.42	3+0.37
Standard	Diclofenac sodium	25mg/kg	2.83+0.31	3.17+0.17	4.67+0.80**	6.67+0.80**	9+0.37***
Test-I	EELL	150mg/k	2.50+0.2	2.67+0.3	2.66+0.22	4.33+0.56	6.33+1.23*

		g	2	3			
Test-II	EELL	300mg/k g	2.00+0.2 0	3.17+0.3 1	5+0.93	6.50+0.89*	8.67+0.67** *

Values are expressed as mean + SEM (n=6); Statistical Analysis of data was carried out by Student t-test comparision test, *p< 0.05, **p <0.01, ***p < 0.001 compared to distilled water treated group; EELL=Ethanolic Extract of Loni ceraligustrina.







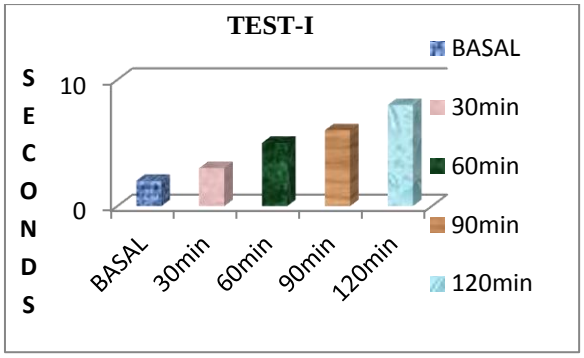


Fig 1: Graphical representation by Tail immersion method using Swiss albino mice.

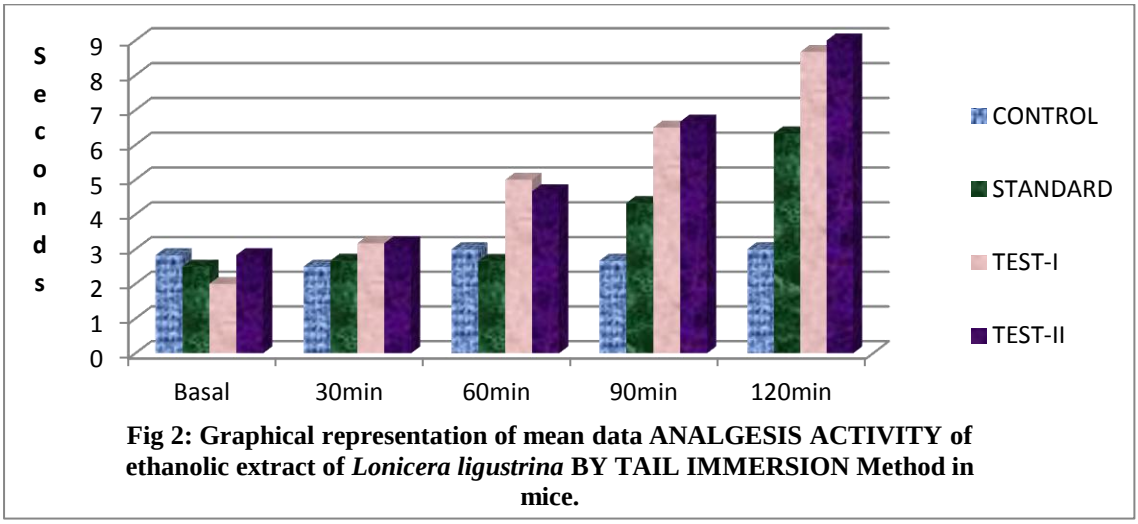


Fig 2: Graphical representation of mean data ANALGESIS ACTIVITY of ethanolic extract of *Lonicera ligustrina* BY TAIL IMMERSION Method in mice.

Table 4: Tail Flick Method

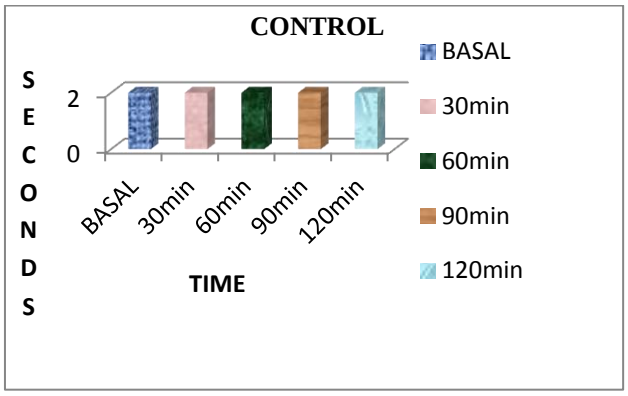
Group	Treatment	Dose (per kg/i.p.)	Swiss albino mice	Reaction time(sec) Post-Administration				
				Basal	30min	60min	90min	120min
Control	Distilled water	10ml	1	2	2	3	2	1
			2	3	2	3	2	4
			3	2	3	2	4	4
			4	3	2	3	4	3
			5	1	3	2	2	2
			6	2	2	3	2	2
Standard	Diclofenac sodium	25mg/kg	1	2	3	6	10	12
			2	3	4	5	8	10
			3	2	3	6	6	6
			4	3	4	3	6	8
			5	4	4	4	4	4
			6	3	6	8	9	8
Test-I	EELL	150mg/kg	1	2	3	6	8	10
			2	3	5	8	8	8
			3	2	3	5	6	8
			4	2	3	2	4	6
			5	2	3	6	8	10
			6	3	3	6	7	9
Test-II	EELL	300mg/kg	1	2	3	3	4	8
			2	3	4	6	8	10

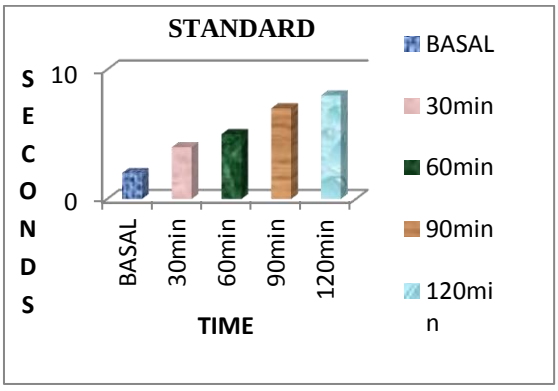
3	2	3	6	8	10
4	1	3	8	9	10
5	2	3	6	8	8
6	2	3	8	9	9

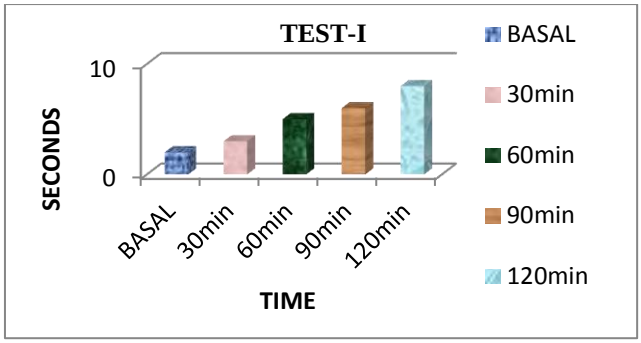
Table 5 : Data of Analgesic Activity of EELL by Tail Flick Method on Swiss Albino Mice

Group	Treatment	Dose (per kg/i.p.)	Reaction time(sec) Post-Administration				
			Basal	30min	60min	90min	120min
Control	Distilled water	10ml	2.17+0.31	2.33+0.21	2.67+0.21	2.67+0.42	2.67+0.49
Standard	Diclofenac sodium	25mg/kg	2.83+0.31	4.00+0.45	5.33+0.71*	7.17+0.9**	8+2.15***
Test-I	EELL	50mg/kg	2.33+0.21	3.33+0.33	5.50+0.81	6.83+0.65*	8.50+0.62***
Test-II	EELL	300mg/kg	2.00+0.26	3.17+0.17	6.17+0.75	7.67+0.76**	9.17+0.40***

Values are expressed as mean + SEM (n=6); Statistical Analysis of data was carried out by Student t-test comparision test, *p<0.05, **p<0.01, ***p<0.001 compared to distilled water treated group; EELL=Ethanolic Extract of Lonicera ligustrina







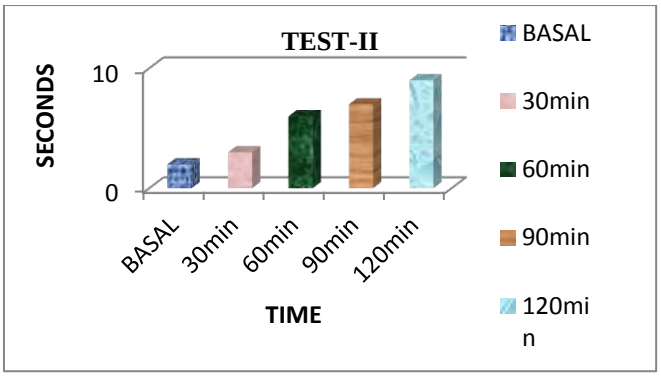


Fig 3: Graphical representation by Tail flick method using Swiss albino mice.

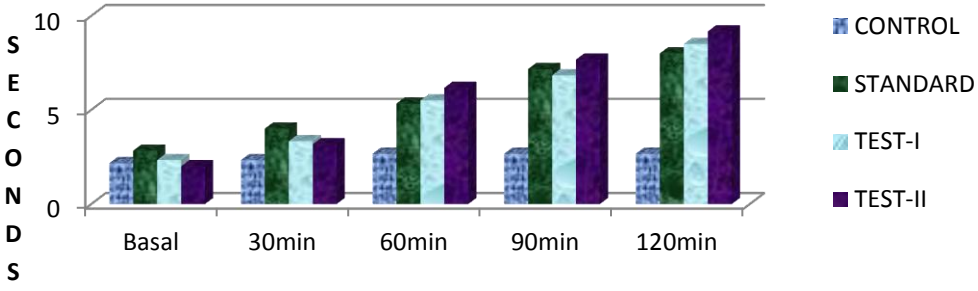


Fig 4: Graphical representation of mean data analgesic activity of ethanolic extract of *Lonicera ligustrina* by Tail -flick method

DISCUSSION & CONCLUSION

In this method there was no significant difference in basal reaction time observed between all the treatment groups indicated that all swiss albino mice have equal sensitivity level to heat. The study was carried out by Tail

Immersion method and Tail flick method. The standard drug dose of Diclofenac sodium (25mg/kg, i.p.) shows siginificant ($p<0.01$ and <0.001) increase in the reaction time after 60 and 90mins respectively. The EELL at a dose level 150 and 300mg/kg, body weight i.p. showed significant ($p<0.05$, $p<0.01$ and $p<0.001$) increase in the reaction time after 60mins when compare to distilled water treatment mice. The analgesic activity of the EELL was comparable with the standard drug Diclofenac sodium, the data shown in tables 3 and 5. and figures 2 and 4. Hence it is concluded that the ethanolic extracts of Loniceraligustrina leaf extract as shown in the table 5.2, shows presence of Flavanoids, Terpenoids, Steroids, Alkaloids, Glycosides, etc. does not shown any morbidity and mortality of mice and shown significant analgesic effect orsignificant increase in a reaction time against thermal stimuli at 60min and 90mins oforal administration respectively and this effect may be attributed through decrease in the pain involvement of biogenic amines like BK, 5-HT,

PGs, NA, NGF, neuropeptides like SP, CGRP and local interferons like enkephalins, etc.
Hence,the further long term study is required to identify, isolate the specific phytochemical drug showing analgesic activity and therapeutic confirmation of EELL leaf extract using different animal models.

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