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Original Article

Pharmacology Section

Clinical Evaluation of Analgesic Activity of Guduchi (*Tinospora Cordifolia*) Using Animal Model

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ABSTRACT

Introduction: Pain is a very well-known signal of ill health and analgesics are the drugs that are used to relieve pain. The main problem with these drugs remains that of side effects. Safer alternatives are natural herbs. Guduchi (*Tinospora cordifolia*) is one such plant with analgesic potential but few studies are there.

Objective: To evaluate the analgesic activity of commercially available extract of Guduchi *(T. cordifolia*).

Materials and Methods: For this purpose commercially available extract of Guduchi (*T. cordifolia*) by Himalaya Drug Company, Bangalore was used. Albino rats were divided randomly in three groups of six rats each. Group 1 (control) received distilled water orally, group 2 (test) received *T. cordifolia* extract in dose of 300 mg/kg orally and group 3(standard) received Pentazocine in dose 10mg/kg intraperitoneally. Analgesic activity was evaluated using hot plate and abdominal writhing method. All the observations were analysed statistically using student's t-test.

Observation and Results: *T. cordifolia* extract significantly ($p<0.05$) increased the response time and decreased the number of writhes in hot plate method and abdominal writhing method respectively, on comparison with the control group.

Conclusions: The above findings suggest that this commercially available extract of Guduchi (*T. cordifolia*) possess analgesic activity. This analgesic activity probably involves peripheral as well as central mechanisms as the extract showed analgesic activity in both hot plate and abdominal writhing method.

Keywords: Anti-nociceptive, Analgesic, Guduchi, Hot plate method, Pain, *Tinospora cordifolia*, Writhing

INTRODUCTION

Guduchi (*Tinospora cordifolia*) [Table/Fig-1] is a member of Menispermaceae family. It is also known as Giloe [1], Giloy, Gurcha (Hindi) and Amrta (Sanskrit). It is found almost everywhere in India and in Himalayas, even up to 1000 feet height. Its habitat ranges across a wide region in India spreading from Kumaon Mountains to Kanyakumari [2]. It is also found in China, Myanmar, Sri Lanka, Thailand, Philippines, Indonesia, Malaysia, Borneo, Vietnam, Bangladesh, North Africa, West Africa and South Africa [2,3].

Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, anti-spasmodic, anti-inflammatory, anti-arthritic, hepatoprotective, anti-allergic and anti-diabetic properties. The plant is used in ayurvedic medicine as "Rasayanas" to improve the immune system and the body resistance against infections. It is also known by the name magical herb due to its property to cure a number of diseases [4].

In a previous study the water extract of the stem of Neem-Giloe [The *T. cordifolia* that grow on Azadirachta indica (neem)] a statistically significant and dose dependent mild analgesic activity. It also potentiated analgesic effect of morphine [5].

Another study showed that the aqueous extracts of *Tinospora cordifolia* (AETC) has significant analgesic and anti-inflammatory activities [6].

In another study, the extract of aerial parts of *Tinospora cordifolia* produced a significant increase in pain threshold in hotplate and tail flick tests in a dose dependent manner. In acetic acid-induced writhing the extract produced significant inhibition of writhing reaction [7].

Freshly prepared extracts are used in the above mention studies for evaluating the analgesic activity. But a crude extract of Tinospora is already available commercially in the market with the brand name "guduchi" which is used as an immunomodulator.

"Guduchi" is a crude extract containing aqueous extracts of both stem and aerial parts of *T. cordifolia*. The activity of plant products vary with the parts used in the extract as each part have different phytochemicals so this extract may or may not possess analgesic activity. So, we wanted to evaluate this commercially available extract for its analgesic activity using animal model.

[Table/Fig-1]: *Tinospora cordifolia* (Guduchi)

Group No.	Group Name	Drug Administered	Dose
1	Control (C)	Distilled Water	0.4ml
2	*Tinospora cordifolia*(TC)	*Tinospora cordifolia* extract	300mg/kg
3	Standard (S)	Pentazocine	10mg/kg

[Table/Fig-2]: Groups of animals (n = 6 for each group)

[Table/Fig-3]: Writhing in rat

MATERIALS AND METHODS

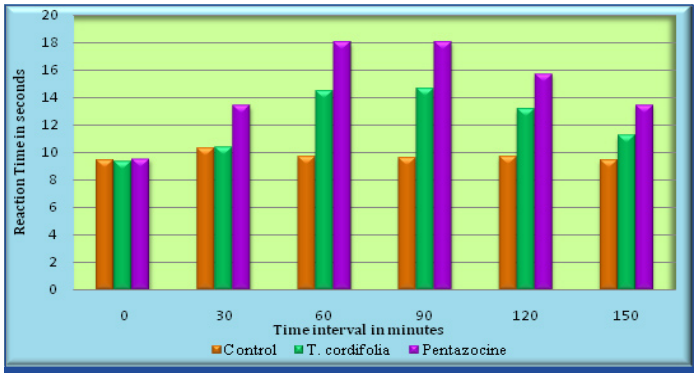
This study was conducted in the Department of Pharmacology Moti Lal Nehru Medical College, Allahabad India. Albino rats of both sexes (male and female) weighing between 100 - 150 gm were used. Albino rats were obtained from registered sellers (Reg. No.- B-37/0605003769) and kept in animal house under the supervision of veterinary doctor. All rats were housed at an ambient temperature of 25°C± 2°C with a 12h light/dark cycle, and provided with standard pellet diet and water adlibitum. The maintenance of the animals was in accordance with the guiding principles of Institutional Animal Ethics committee and the Guide for the Care and Use of Laboratory. Animals published by the National Institute of Health (NIH Publication. No. 85-23 revised 1996, Latest revision in 2011). All the experimental procedures and protocols used in the study were reviewed and approved by Institutional Ethics Committee (Approval No. IEC/MLNMC/2013/No.11).

Test Drugs and Chemicals

All the drugs were administered orally with the help of feeding tube after preparing suspension in distilled water (vehicle).

Mean latency (seconds) ± S.D at time interval (minutes)						
Groups	0	30	60	90	120	150
Control	9.40±2.07	10.30±2.34	9.64±2.42	9.59±2.36	9.63±1.73	9.42±2.19
T. cordifolia	9.35±2.15	10.32±2.62	14.48±2.61*	14.64±2.60*	13.11±2.13*	11.18±2.32
Standard	9.50±2.14	13.38±1.30*	17.99±1.37*	18.03±1.15*	15.68±1.53*	13.41±1.81*
(*p-value<0.05)						

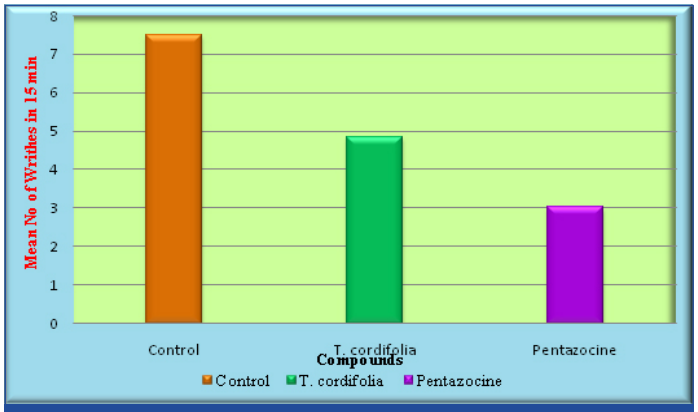
[Table/Fig-4]: Effect of *T. cordifolia* on hot plate latency in groups



[Table/Fig-5]: Bar diagram showing comparison of mean hot plate latency (seconds) of Control group, *T. cordifolia* extract group and Pentazocine group at various time interval

Groups	No. of Writhes	% Inhibition
Control	7.50±1.05	--
T. cordifolia	4.83±0.75*	35.6
Standard	3.00±0.89*	60

[Table/Fig-6]: Effect of *T. cordifolia* on abdominal writhing



[Table/Fig-7]: Bar diagram representing mean no. of abdominal writhes

Tinospora cordifolia extract

It was procured as commercially available capsules with brand name "guduchi" from the Himalaya Drug Co., Bangalore, India. Each capsule contain crude extract in dry powder form. The extract was given in a dose of 300mg/kg orally [6-8].

Pentazocine

It was obtained from Neon Laboratories, Mumbai. It was given in dose 10 mg/kg by intra peritoneal route [9].

Sodium Chloride

It was obtained from s. d. fine-chem Ltd. Boisar.

Experimental Protocol

Eighteen albino rats were taken after screening by hot plate for abnormal response (latency > 30s excluded). Then these rats were divided randomly (using random number table) into three groups of six rats each [Table/Fig-2].

Analgesic Activity

The analgesic activity was evaluated using hot plate and abdominal writhing method.

Hot Plate Method

The method, originally described by Woolfe and Mac Donald [10] has been modified by several investigators. The animal was placed on the hot plate, maintained at 55 ±1°C temperature, and the time until either licking of paw or jumping occurs was recorded by a stop-watch. The latency was recorded before and 30, 60, 90, 120 and 150 minutes after the administration of the control, standard or the test compound. A cut off period of 30 seconds was observed to avoid damage to the paws. The values of the reaction time of experimental groups were compared with that of the control group. The prolongation of the latency times indicated the anti-nociceptive activity.

Abdominal Writhing Test

The writhing phenomenon in rats was demonstrated by Fukowa

et al., [11]. Pain is introduced by injection of irritants into peritoneal cavity of rats. The animals react with a characteristic stretching behaviour which is called writhing. The control, test or the standard compound were administered to the test animals. Forty five minutes later 6% sodium chloride solution [12] was injected intraperitoneally then each rat was placed individually into glass beakers and the number of writhes occurring between 5 to 20 minutes after sodium chloride injection was counted for each animal. For scoring purposes, a writhes is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb [Table/Fig-3]. The formula for computing per cent inhibition is:

(number of writhes in control group-number of writhes in test group)/(number of writhes in control group)×100

STATISTICAL ANALYSIS

The observations were analysed using one-way ANOVA and student t-test where ever needed and p-value less than 0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

The study was carried out on albino rats of either sex weighing 100-150g. Experimental pain models, response to thermal stimulation by hot plate and abdominal writhing, were used for assessing the analgesic effects in rats. The test compound was administered orally, while the standard drug Pentazocine was administered intraperitoneally.

Hot Plate Method

The mean hot plate latency of all three groups, before administration of compound (0 minute), was compared using ANOVA which revealed similar mean baseline hot plate latency among the groups ($f=0.008$, $p>0.05$).

Administration of this *T. cordifolia* extract (300mg/kg) orally produced statistically significant analgesia when compared with the control group at 60 minutes ($p<0.01$), 90 minutes ($p<0.01$) and 120 minutes ($p<0.05$) while administration of Pentazocine (10mg/kg) produced statistically significant analgesia when compared with the control group at 30 minutes ($p<0.05$), 60 minutes ($p<0.001$), 90 minutes ($p<0.001$), 120 minutes ($p<0.001$) and 150 minutes ($p<0.01$) [Table/Fig-4,5]. Peak effect of both substances occurred 90 minutes after the administration of compound

These results indicate that this commercially available extract of *T. Cordifolia* produced statistically significant ($p<0.05$) analgesia when compared with the control group in hot plate method.

Abdominal Writhing Method

Administration of this *T. cordifolia* extract orally and Pentazocine intraperitoneally significantly reduce writhes counts ($p<0.001$ & $p<0.001$ respectively) when compared with the control group [Table/Fig-6,7]. Therefore, this commercially available extract of *T. Cordifolia* produced a statistically significant analgesia in Sodium Chloride (NaCl) induced abdominal writhing method.

DISCUSSION

The group of rats receiving *Tinospora cordifolia* extract showed statistically significant difference with the control group at 60 minutes ($p<0.01$), 90 minutes ($p<0.01$) and 120 minutes ($p<0.05$) in hot plate method and produced statistically significant decrease in writhes count when compared with the control group ($p<0.001$). These results show that this extract possess a significant analgesic activity.

The hot plate method is considered to be selective for the drugs acting centrally. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity [13]. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [14].

The abdominal writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway [15,16].

Though, it is not the scope of the study to elucidate the mechanism of action of this extract buton the basis of previous studies the probable mechanism of action of this extract as analgesic are

It is possible that the extract acted on opioid receptor [4]. It also potentiated analgesic effect of morphine so its activity might involve opioid receptors [5].

Antioxidant and calcium attenuating actions of aqueous and alcoholic extract of *Tinospora cordifolia* is contributing for attenuating sciatica pain associated with sciatic nerve root ligation [17].

Petroleum ether extract of *T. cordifolia* found to increase the levels of monoamines like noradrenaline, serotonin, and dopamine [18] which may be responsible for its anti-nociceptive activity.

T. cordifolia contains alkaloids, glycosides, flavonoids, steroids and terpenoids in the aerial part of the plant. So, the observed analgesic activity may be attributed to any of these phytoconstituents. There are also reports of analgesic activity of flavonoid which is mediated by inhibiting the production of prostaglandins [4].

Prostaglandins sensitises the peripheral nerve endings. Moreover, substances like bradykinin, Substance P (potent mediators of pain) are released during inflammation. Anti-inflammatory agents like NSAIDs are used as analgesics and decrease pain by decreasing various mediators of inflammation especially PG.

Gastric irritation is a common side effect of NSAIDs while Tinospora possesses gastro protective activity [2] which is a beneficial property while using it as an analgesic.

After the completion of the study, a few points that need to be pondered over are:

- Studies with still higher doses and longer duration are needed to be done, so as to define the maximum effective doses and toxic doses.
- In the study, inclusion of a standard NSAID would have provided a better comparison of the anti-nociceptive activity in abdominal writhing method.
- The results might come out to be of a better magnitude, if the active principles of the test compounds are used for the study instead of the crude extract.

CONCLUSION

Commercially available extract of *T. cordifolia* "guduchi" was found to possess analgesic activity. As it showed analgesic activity in both the methods so its analgesic activity might involve central (opioid receptors) as well as peripheral (inhibition of PG synthesis) mechanisms.

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