ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Clinical Evaluation of Anti-Nociceptive Activity of *Terminalia arjuna* extract In Albino Rats.

Bhomik Goel[1]*, Nishant Pathak[1], Dwividendra Kumar Nim[2], Rakesh Kumar Dixit[3], and Rakesh Chaurasia[4]

[1]Junior Resident, Pharmacology, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India.
[2]Senior Resident, Pharmacology, LHMC, New Delhi, India.
[3]Professor, Pharmacology, KGMU, Lucknow, Uttar Pradesh, India.
[4]Associate Professor, Pharmacology, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India.

ABSTRACT

The main aim of the study was to evaluate the anti-nociceptive activity of *Terminaliaarjuna*. Commercially available extract of *T. arjuna* was used**.** Albino rats were divided randomly in 3 groups of 6 rats each. Group 1 (control) received distilled water orally, group 2 (test) received *T. arjuna* extract in dose of 800 mg/kg orally and group 3(standard) received Pentazocine in dose 10mg/kg intraperitoneally. Anti-nociceptive activity was evaluated using hot plate and abdominal writhing method *T. arjuna* 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. The above findings suggest that *T. arjuna* extract possess anti-nociceptive activity. This anti-nociceptive activity probably involves peripheral(inhibition of co) as well as central mechanisms

Keywords Terminalia arjuna, anti-nociceptive, pain, analgesic, hot plate method

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Pain is a very well-known signal of ill health that is felt by all but still very difficult to define. It is the most common reason for physician consultation, a major symptom in many medical conditions, and can significantly interfere with a person's quality of life and general functioning [1]. Analgesics are the drugs that are used to achieve relief from pain. They include a number of drugs like Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as salicylates, opioids such as morphine. But side effects limit their use. So world is in a constant need of newer and safer analgesics.

Terminalia arjuna is a member of *Combretaceae* family commonly known as Arjuna or Arjun treein English. It is a large evergreen tree whose different parts namely fruits, leaves and stem bark are used in Ayurveda, Siddha and Unani system. Different parts of this tree are used for treatment of various conditions. Bark is used for treatment and prevention of various cardiovascular diseases, treatment of wounds, haemorrhage and ulcers. In combination with other herbs Arjun is used for the treatment of gout, cough and pain.

Only few studies were conducted to evaluate the anti-nociceptive activity of *Terminalia arjuna* so the purpose of the present study was to evaluate the anti-nociceptive activity (if it have any) of*Terminalia arjuna* in rat models of pain.

MATERIALS AND METHODS

This study was conducted in the Departmentof Pharmacology Moti Lal Nehru Medical College, Allahabad. Albino rats of both sexes (maleand female) weighing between 100 - 150 gm. were used. Albino rats were obtained from registeredsellers (Reg. No.- B-37/0605003769) and keptin animal house under the supervision ofveterinary doctor. All rats were housed at anambient temperature of 25^{o}C± 2°C with a 12hour light/dark cycle, and provided withstandard pellet diet and water *adlibitum.* The maintenance of the animals was inaccordance with the guiding principles ofInstitutional Animal Ethics committee and theGuide for the Care and Use of Laboratory. Animals published by the National Institute ofHealth (NIH Publication. No. 85-23 revised1996, 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 thehelp of feeding tube after preparingsuspension in distilled water (vehicle).

Terminalia arjunaextract

It was procured as commercially availablecrude extract in dry powder form, from the Himalaya Drug Co., Banglore, India. It was given in a dose of 800mg/kg orally [2].

Pentazocine

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

Sodium chloride

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

Experimental Protocol

18 albino rats were taken and screened by hot plate for abnormal response (latency > 30s). Then these rats were divided randomly (using random number table) into 3 groups of 6 rats each (table 1).

Table 1. Groups of animals (n = 6 for each group)

Group No.	Group Name	Drug Administered	Dose
1	Control (C)	*Distilled Water*	0.4ml
2	*Terminalia arjuna* (TA)	*Terminalia arjuna extract*	800mg/kg
3	Standard (S)	*Pentazocine*	10mg/kg

Anti-nociceptive Activity

The anti-nociceptive activity was evaluated using hot plate and abdominal writhing method.

Hot Plate Method

The method, originally described by Woolfe and Mac Donald [3] 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*. [4]. 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 [5] 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 theabdomenwith simultaneous stretching of at least one hind limb. The formula for computing per cent inhibition is:

$$\frac{(number\ of\ writhes\ in\ control\ group - number\ of\ writhes\ in\ test\ group\ )}{number\ of\ writhes\ in\ control\ group} \times 100$$

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 in 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 3 groups, before administration of compound (0 minute), was compared using ANOVA which revealed similar mean baseline hot plate latency among the groups ($F=0.128$, $p>0.05$). Results of hotplate test are presented in table 2.*T. arjuna* extract produced statistically significant ($p<0.05$) analgesia when compared with the control group.

Table 2. Hot plate latency in groups

Groups	Mean latency (seconds) ± S.D at time interval (minutes)					
	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. arjuna	8.93±2.06	10.07±1.86	14.79±1.94*	15.45±1.52*	13.17±1.87*	10.57±1.75
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)

Abdominal Writhing Method

Terminalia arjuna extract produced a statistically significant reduction in 6% NaCl induced abdominal writhing ($p<0.01$). Results are presented in table. 3

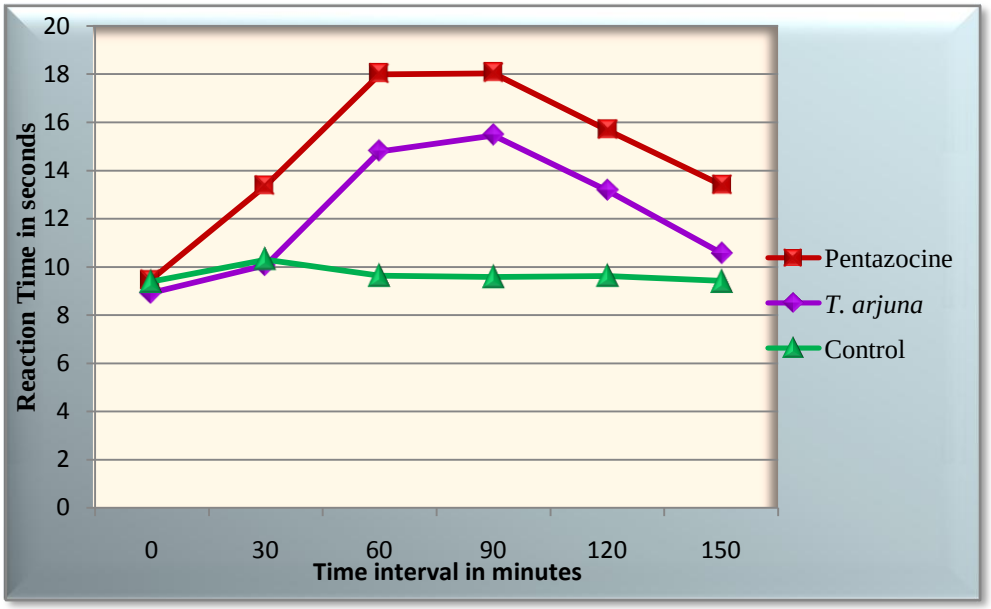
ISSN: 0975-8585

Table 3. Effect of *T. arjuna* on abdominal writhing

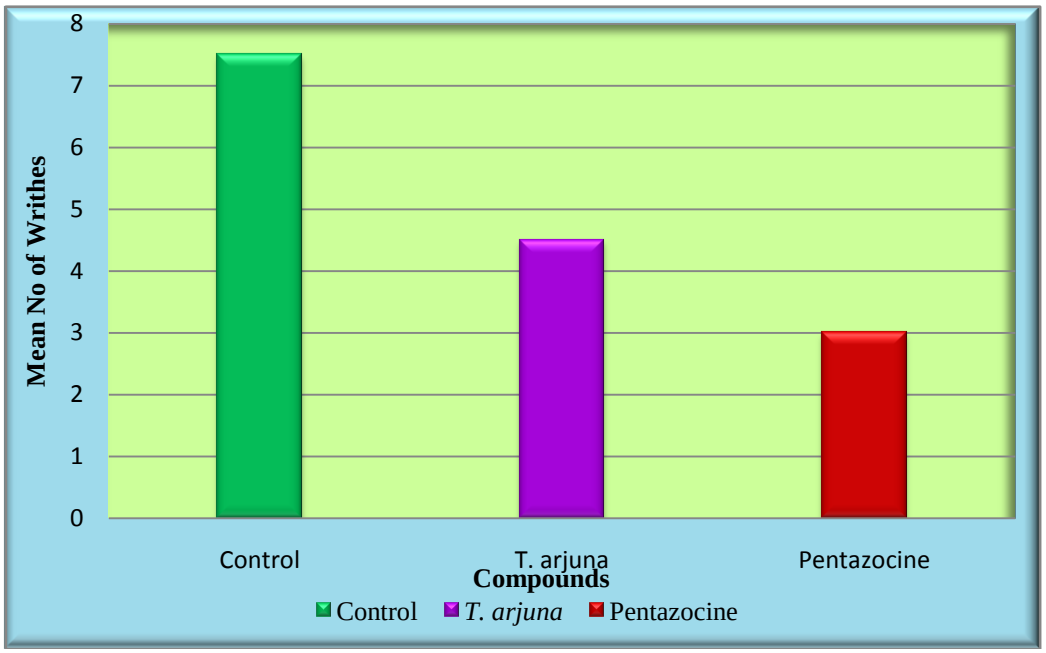
Groups	No. of Writhes	% Inhibition
Control	7.50±1.05	
T. arjuna	4.50±1.05*	40
Standard	3.00±0.89*	60

*(*p-value<0.05)*

Graph 1: Line diagram showing hot plate latency



Graph 2: Bar diagram representing Mean No. of abdominal writhes



ISSN: 0975-8585

DISCUSSION

The hot plat method is considered to be selective for the drugs acting centrally. The hot plat 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 [6]. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [7]. Therefore, the extract of the plant must have a central activity.

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 [8,9]. Therefore, it may be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway. The plant extract of *T. arjuna*exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism [6].

Naloxone an opioid antagonist abolished *T. arjuna*inducedantinociception in tail flick model. These results suggest that *T.arjuna* exhibits antinociceptive activity by central effect probably mediated via central opioidreceptors or by promoting the release of endogenousopiopeptides [2].

In the central regions involving the supra-spinal andspinal control of nociception, such as thalamus and spinal cord, the colocalisation of μ opioid receptors and nAChRs have been reported [10]. A close relationshipbetween opioid and cholinergic mechanism has beenobserved in relation to the augmentation of the releaseand biosynthesis of endogenous opioid peptides [11]. *T.arjuna* might be acting on any of these receptors andaugmenting the release of endogenous opioid peptides.

Preliminary qualitative phytochemical screening reveals the presence of alkaloids, carbohydrates, tannins, gums, terpenoids & flavonoids in *T. arjuna*. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [12,13]. There are also reports on the role of tannins in anti-nociceptive activity [14]. Besides alkaloids are well known for their ability to inhibit pain perception [15], Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with anti-phlogistic activity [16].

The exact mechanisms of activity are not yet very well elucidated. The proposed mechanisms need to be explored further through extensive studies.

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

- Studies with still higher doses need 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.

- The results might come out to be of a better magnitude, if the active principle of the test compound isused for the study instead of the crude extract.

CONCLUSIONS

Since the plant extract significantly reduced the number of writhes in abdominal writhing model and increased hot plate latency in hot plate model, the commercially available crude extract of Terminalia arjuna exhibit anti-nociceptive activity. More studies are needed to elucidate their optimumdose, exact mechanism of action.

REFERENCES

[1] Breivik H, et al. Br J Anaesth 2008; 101(1):17–24.
[2] SumitaHalder NB, Pramod K Mediratta, Inderjeet Kaur, Krishna K Sharma. Indian J Exp Biol 2009;47:577-83.
[3] Woolfe G, MacDonald AD. J Pharmacol Exper Ther 1944; 80: 300–307
[4] Fukawa K, Kawano O, Hibi M, Misaki M, Ohba S, Hatanaka Y. J Pharmacol Meth 1980; 4: 251–259
[5] VonVoigtlander PF, Lewis RA. J Pharmacol Meth 1983; 10: 277–282.
[6] Sabina EP, Chandel S and Rasool MK. International Journal of Integrative Biology. 2009; 6(2): 52-56.
[7] Ibironke GF. And Ajiboye KI. Int J Pharmacol 2007; 3: 111-115.
[8] Duarte, IDG., Nakamura, M. and Ferreira, SH. Brazilian J Med Biol Res 1988; 21: 341–343.
[9] Derardt R, Jougney S, Delevalccee F and Falhout M. European J Pharmacol 1980; 51: 17-24.
[10] Mansour A, Fox C A, Akil H & Watson SJ. Trends Neurosci 1995;18:22.
[11] Houdi A A, Pierzchala K, Marson L, Palkovits M and Van Loon GR. Peptides 1991;12:161.
[12] Rajnarayana K, Reddy MS, Chaluvadi MR and Krishna DR. Indian J Pharmacol 2001; 33:2-16.
[13] Rao MR, Rao YM, Rao AV, Prabhkar MC, Rao CS and Muralidhar N. J Ethnopharmacol 1998; 62: 63-66.
[14] Vanu MR, Palanivelu S and Panchanatham S. Biol Pharm Bull 2006; 29: 693-700.
[15] Uche, FI. andAprioku, JS. J App Sci Environ Man 2008; 12(4): 99–102.
[16] Wagner H. Planta Medica 1989; 55: 235-241.