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Pharmacological Study

Evaluation of analgesic activity of *Terminalia arjuna* (Roxb.) Wight and Arn bark: A tribal claim

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Abstract

Background: Plants occupy an important place in folk medicine all over the world for centuries and indigenous communities have developed their own specific knowledge on plant resources, uses, management, and conservation. Research interest and activities in the area of ethno medicine have increased tremendously in the last decade. Currently, scientists are evincing keen interest in the scientific evaluation of ethno medical claims. Bark powder of *Arjuna* (*Terminalia arjuna* [Roxb.] Wight and Arn) is used by tribals for the management of some painful conditions. **Aim:** To evaluate analgesic activity of *T. arjuna* bark in rodents. **Materials and Methods:** For evaluation of analgesic activity, different experimental models, that is, the acetic acid-induced writhing syndrome in mice, formaldehyde-induced paw licking response and tail flick test in rats were designed. Experiments were carried out at two-dose levels, that is, therapeutically equivalent dose (TED) and TED × 2. Animals were divided into three groups (six animals in each group), first group serving as a control group, second and third group labeled as test drug group. **Results:** Test drug at both the doses significantly decreased the writhing syndrome in comparison to control the group. In comparison to control the group, incidences of formalin-induced paw licking were reduced in test drug groups in both early and late phases of pain. In tail flick response, threshold was significantly increased in both test drug groups at every time intervals. **Conclusion:** Study showed that stem bark of *T. arjuna* possesses analgesic activity in all experimental models.

Key words: *Arjuna* bark, pain, reverse pharmacology

Introduction

Terminalia arjuna (Roxb.) W. A. (Family: Combretaceae), is a source plant of Ayurvedic classical drug namely *Arjuna*. Charaka Samhita included *Arjuna* among the groups indicated for the management of *Udarda* (urticaria).[1] Vrindamadhava (9th AD), a medieval compendium recommended it in the management of *Hridroga* (heart disease).[2] The drug is attributed with wound healing property by most popular *Nighantus* like Dhanvantari, Kaiyadeva, Raja, and Bhavaprakash.[3] *Hridya* (cardiotonic) activity was described by Bhavamishra.[4] *Arjuna* is frequently employed by tribals in the management various diseased conditions.

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Tribal claims include

Five gram powder, made of equal quantities of stem bark of *T. arjuna*, *Pterocarpus marsupium*, and flowers of *Cassia occidentalis* are mixed in 100 ml of hot milk and given once a day for a month or two, to save the patient from tuberculosis.[5] Leaf paste of *T. arjuna* is used like soap nut to get rid of dandruff.[6] Twenty gram of stem bark of *T. arjuna* in 50 ml of water is boiled to half, filtered, mixed in 50 ml of goat milk and given internally, once a day for a week, to control palpitation and high-blood pressure.[7] Five gram of finely powdered stem bark of *T. arjuna* is administered twice a day for 3 days to relive pain in lower limbs.[8]

Terminalia arjuna is a large deciduous tree, commonly found throughout the greater parts of the country. Bark available in pieces, flat, curved, recurved, channeled to half quilled, 0.2–1.5 cm thick, market samples up to 10 cm in length and up to 7 cm in width, the outer surface somewhat smooth and grey, the inner surface somewhat fibrous and pinkish, transversely cut smoothened bark shows pinkish surface; fracture - short

in inner and laminated in the outer part; taste is bitter and astringent.[9]

Certain experimental studies provided evidence for its hypotensive activity in dogs,[10] protection against isoproterenol induced myocardial ischemic injury in Wistar albino mice and hypolipidemic activity in hyperlipidemic rabbits.[11] Some of the studies confirmed antibacterial, antiviral, and wound healing activities.[12-14] Clinical studies documented beneficial activity in patients suffering from congestive cardiac failure.[15] A critical review of research studies so far carried out on *Arjuna* clearly indicates that the bark of it was not studied for its analgesic activity. Keeping this in view, the present study was planned and carried out based on tribal claim for assessing analgesic activity in Swiss albino mice by tail flick method, acetic acid-induced writhing reflex and formaldehyde-induced paw licking method.

Materials and Methods

Collection of plant materials

The bark of *T. arjuna* was collected from outskirts of Jamnagar, Gujarat and was authenticated by expert, Pharmacognosy Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar. Sample was deposited in the herbarium of Pharmacognosy Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar. The flat thick bark was made into pieces, and shade dried for 15 days and then pulverized. Powder was stored in air tight glass container to carry out further evaluation.

Experimental animals

Charles foster strain albino rats of either sex; weighing 200 ± 20 g for formaldehyde-induced paw licking response and Swiss albino mice of either sex, weighing 30 ± 6 g for the acetic acid-induced writhing syndrome and tail flick test were used for the study. Animals were obtained from the animal house attached to the Pharmacology Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar. The animals were maintained on "Amrut" brand animal pellet feed of Pranav Agro Industries and tap water was given *ad libitum*. The temperature and humidity were kept at optimum level and animals were exposed to natural day-night cycles. The experiments were carried out in conformity with the guidelines of the Institutional Animal Ethics Committee (IAEC) after obtaining its permission (approval no. IAEC/10/12/21) and care of animals was taken as per the Committee's guidelines for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Dose fixation and grouping

The dose fixation for the experimental animals was done on the basis of body surface area ratio by referring to the standard table of Paget and Barnes.[16] The adult human dose (3 g/day) was converted to animal dose. On this basis, the rat dose and mice dose were found to be 270 mg/kg and 400 mg/kg, respectively. All experiments were carried out at two-dose levels, viz., therapeutically equivalent dose (TED) and TED × 2. For each study, 18 animals were selected and divided into three groups containing equal animals wherein first group serving as a control group receiving distilled water only and second and third group labeled as TED and TED × 2 groups. The test drug was suspended in deionized water and administered orally with the help of gastric catheter sleeved to syringe.

Acetic acid-induced writhing syndrome

Acetic acid (1% v/v) was administered intraperitoneally to all the groups at a dose of 1 ml/kg body weight 60 min after the administration of test compounds.[17] The analgesic effect was recorded by counting the number of writhes after the injection of acetic acid for a period of 30 min. A writhe is indicated by abdominal constriction and full extension of the hind limbs.

Formaldehyde-induced paw licking response in rats

The effect of test drug on the formaldehyde-induced paw licking response was evaluated by adopting the method used in previous research work.[18] After the injection of formaldehyde, the animals were kept under observation for half an hour. The time taken for the onset of paw licking, and its frequency was measured in two phases as 0–10 min and 20–30 min.

Tail flick test

The basal reaction time of animals to radiant heat was recorded by placing the tip (last 1–2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. The animals that showed a flicking response within 3–5 s were selected for the study. A cut-off period of 15 s is observed to avoid damage to the tail. After the administration of the drug, the tail flick response was taken at 30 min, 60 min, 90 min, 120 min, 180 min, and 240 min.[19]

Statistical analysis

Student's *t*-test for unpaired data has been used for analyzing the data generated during the study.

Observations and Results

Study on acetic acid-induced writhing syndrome in mice showed that pretreatment with test drug at both the dose levels apparently decreased the latency of onset of writhing. However, only the decrease observed in onset of writhing in TED treated group is found to be statistically significant. Test drug at both the doses significantly decreased the writhing syndrome in comparison to control group [Table 1].

In comparison to control group, incidences of formalin-induced paw licking were reduced in test drug groups at both the dose levels in both early and late phases of pain. However, only the inhibition of pain observed in late phase nociception is found to be statistically significant in both test drug groups [Table 2].

In tail flick response, threshold for tail flick response was significantly increased in both test drug-treated groups at every time intervals in comparison to control group. The observed increase for tail flick response in TED dosed group is found to be better than that of high dose treated group [Table 3].

Discussion

Intraperitoneal administration of acetic acid releases prostaglandins and phlogistic mediators like PGE2 and PGE2a, and their levels were increase in the peritoneal fluid of the acetic acid-induced mice.[20] The drug in the therapeutic

Table 1: Effect on the acetic acid-induced writhing syndrome in mice

Groups	Dose (mg/kg)	Latency of onset		Frequency of writhing for 30 min after acetic acid injection	
		Onset (s)	Percentage of change	Number of syndrome	Percentage of change
Control	Q.S.	178.80±25.50	-	45.33±4.01	-
TED	400	35.58±8.68***	80.10↓	12.33±3.10***	72.80↓
TED×2	800	152.20±29.38	14.88↓	07.50±2.67***	83.45↓

Data: Mean±SD, ***P<0.001, ↓: Decrease. SD: Standard deviation, TED: Therapeutically equivalent dose

Table 2: Effect on paw lickings response in albino rats

Groups	Dose (mg/kg)	Number of paw lickings			
		0-10 min	Percentage of inhibition	20-30 min	Percentage of inhibition
Control	Q.S.	32.83±3.74	-	30.83±4.06	-
TED	270	24.00±5.55	26.89↓	15.80±2.75*	48.75↓
TED×2	540	24.60±3.04	25.07↓	17.40±1.86*	43.56↓

Data: Mean±SD, *P<0.05, ↓: Decrease. SD: Standard deviation, TED: Therapeutically equivalent dose

Table 3: Effect on tail flick in Swiss albino mice

Group	Dose (mg/kg)	Initial	30 min	60 min	90 min	120 min	180 min	240 min
Control	Q.S.	2.60±0.28	2.32±0.22	2.73±0.21	2.80±0.34	2.18±0.21	2.42±0.19	2.47±0.39
TED	400	2.75±0.25	3.58±0.52*	3.98±0.45*	4.38±0.40*	3.52±0.65	5.03±0.85**	3.90±0.84
TED×2	800	3.34±0.48	3.36±0.64	3.77±0.71	3.83±0.50	3.29±0.58	4.04±0.46**	3.43±0.79

Data: Mean±SD, *P<0.05, **P<0.01, SD: Standard deviation, TED: Therapeutically equivalent dose

dose and in the double dose group significantly ($P < 0.001$) reduced the number of abdominal constrictions and stretching of the hind limbs induced by the injection of acetic acid in a dose-dependent manner. The abdominal constrictions produced after the administration of acetic acid are related to sensitization of the analgesic receptors to prostaglandins. It is, therefore, possible that the drug is effective due to its analgesic effect, probably by inhibiting the synthesis or action of prostaglandins.

When formalin is injected subcutaneously into the paw, it produces intense pain reaction. The effect is seen in two phases. The initial phase lasts for 0–10 min of the formaldehyde injection, it is supposed to be mediated through modulation of neuropeptides.[21] The second phase, which is observed 20–30 min of the formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin. Centrally acting drugs inhibit both phases, while peripherally acting drugs only inhibit the second phase. Test drug at both dose levels insignificantly decreased the paw licking episodes at first phase while significantly decreased at later phase which indicates analgesic activity of test drug seems to be through central mechanism.

Tail flick model, which is thermal-induced nociception indicates narcotic involvement which is sensitive to opioid μ receptors.[22] The study shows prolonged analgesic effect of the test drug. The presence of analgesic activity in this model indicates that the mechanism of action is central. The mechanism through which this effect is brought about may be due to modulation of opioid receptors or by release of endogenous analgesic factors such as encephalin and endorphin.

Conclusion

A tribal claim about powdered stem bark of *Arjuna* (*T. arjuna*) indicated in the management of pain was evaluated in animals (rats and mice) for its analgesic activity against acetic acid-induced writhing syndrome, formaldehyde-induced paw licking response and tail flick test. The study was carried in three groups, that is, control, TED, and TED × 2 groups. Statistically significant analgesic activity was observed in acetic acid-induced writhing syndrome and formaldehyde-induced paw licking response with both TED and TED × 2 groups. In tail flick test, TED group has shown statistically significant response in comparison to TED × 2 groups. The present study has produced scientific validation of the tribal claim which may promote to explore its value by well-planned clinical trials.

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हिन्दी सारांश

अर्जुनत्वक् के वेदनाशामक कर्म का प्रायोगिक अध्ययन

अनुराग गुप्ता, के. निष्ठेश्वर, विनय जे. शुक्ला, बी.के. अशोक

संपूर्ण विश्व में कबीलाई औषधियों में पौधों का शताब्दियों से महत्त्वपूर्ण स्थान है तथा इन लोगों ने पौधों के विभिन्न प्रयोगों के लिये विशिष्ट तरीकों का विकास किया है। पिछले कुछ दशकों में नूतन औषधियों की खोज ने वैज्ञानिकों का ध्यान अपनी ओर आकृष्ट किया है। अर्जुन वृक्ष के तने की छाल का वेदना निवार्णार्थ कबीलाई लोगों द्वारा प्रयोग किया जाता है। इसको वैज्ञानिक आधार प्रदान करने हेतु कुछ पूर्व स्थापित परीक्षण मानकों का प्रयोग किया गया तथा इस प्रयोग में परीक्षित औषध के प्रयोग के परिणाम वेदना शामक सिद्ध हुये है।