



EVALUATION OF SURFACE ANAESTHETIC ACTIVITY OF ALCOHOLIC EXTRACT OF FRUIT OF *TERMINALIA CHEBULA* ON THE CORNEA OF ALBINO RABBITS

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Article Info

Received: 09/04/2013

Revised from:10/04/2013

Accepted on:01/05/2013

ABSTRACT: Dry fruit of *Terminalia chebula* has laxative, astringent, anti-helminthic, expectorant, tonic, carminative, and appetite stimulant effect. It is used in leprosy, anemia, narcosis, piles, chronic, intermittent fever, heart disease, diarrhea, anorexia, cough and excessive secretion of mucus. This study is to see whether the *Terminalia chebula* has local anesthetic activity as it is used for throat pain as lozenge in traditional medicine. Alcoholic extraction of fruit of *Terminalia chebula* (AETC) done by Soxhelt's apparatus. Forty male Rabbit's were taken and divided into four groups each group contains 10 animals, Group I - Standard (2% xylocaine), Group II - Test 0.25% *Terminalia chebula* extract. Group III - Test 0.5% *Terminalia chebula* extract. Group IV - Test 1% *Terminalia chebula* extract was instilled in conjunctival sac of right eyes. Standard protocol was followed to elicit light reflex, corneal reflex and to measure pupillary size after instilling the test drugs in the eye. There was dose dependent increase in onset and duration of local anesthetic activity with different doses of alcoholic extract of fruit of *Terminalia chebula*.

Key words: Corneal reflex, Light reflex, Local anesthetic, Soxhelt's apparatus, *Terminalia chebula*, Xylocaine.

INTRODUCTION

Terminalia chebula (Family: Combretaceae) is a middle-sized tree, leaves are ovate, or Elliptic, flowers are yellowish white; fruits are yellowish brown in colour distributed throughout India [1, 2] and were used extensively in traditional medicine. It has Antibacterial, Antifungal, Antiviral, Anticarcinogenic, Antioxidant, Adaptogenic and Antianaphylactic, Hypolipidemic, Hepatoprotective, Cardio protective, Antidiabetic, Wound healing, Immunomodulatory and Chemo preventive actions [3]. The plant has been used extensively in Ayurveda and Siddha medicine for Constipation, diarrhea, ulcers, gastroenteritis, asthma, cough, dyspnea, dyspepsia, hemorrhoids, candidiasis, parasites, malabsorption syndrome, hepatomegaly, vesicular and renal stones, tumors, skin diseases, leprosy, intermittent fever, rheumatism, arthritis, gout, neuropathy, paralysis, memory loss, epilepsy, depression, diabetes, cardiovascular diseases, anorexia and wounds [4,5] As a home remedy it is advised by elders to keep a piece of *Terminalia chebula* fruit in the mouth as lozenge to suppress irritable cough which may be due to its local anesthetic action. So the

experiment is taken to evaluate local anesthetic action of *Terminalia chebula* fruit.

Material and Methods

Plant Collection: The fruits of *Terminalia chebula* were purchased from a noted and authenticated Ayurvedic shop during the month of February 2013.

Preparation of extract: The fruits of *Terminalia chebula* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material- obtained was then subjected to successive extraction by Hot Percolation Method using methanol solvents in a Soxhelt's extractor. The extract obtained was evaporated at 45°C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of Alcoholic extract was found to be 37.50%w/w and the extract was used for further studies. The study was performed between the months of February 2013 and March 2013, in the department of pharmacology, Mamata Medical College (MMC), Khammam, Andhra Pradesh.

Animals used : Forty albino rabbits of either sex weighing between 2.5-3kg were obtained from central animal house

of MMC. Approval of Institutional Animal Ethics Committee for the experimental protocol was taken. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were housed in Poly propylene cages and maintained at $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under 12h light/ dark cycle and were fed with standard pellet diet and had free access to water.

Drugs used: Xylocaine 2% Plain Injection Manufactured by AstraZeneca, Alcoholic extract of fruit of *Terminalia chebula*

Preparation of doses of Alcoholic extract of *Terminalia chebula* (AETC): 0.25% AETC, 0.5%AETC and 1%AETC prepared from the stock solution. Commercially available 2% Xylocaine is used as standard.

Delivery of the test solutions to experimental groups: The animals were placed in rabbit holding cages and randomly allocated into four groups. The upper and lower eye lashes were carefully clipped off to avoid the corneal reflex

initiated by accidental touching of the eye lashes. Left eye serves as control and right eye as test. The conjunctival sac of right eye held open to form a pocket. In to these pockets Group 1 animals were delivered one drop of 2% Xylocaine. Group11, Group III and Group IV were delivered one drop of 0.25% AETC, 0.5%AETC and 1%AETC respectively. The corneal reflex was tested by touching cornea from the side using a wet cotton wisp. The test was started five minutes after application of the drug in all groups and repeated every five minutes until corneal reflex was lost (blinking lost) followed by reappearance of corneal reflex(blinking reappearance). The time between disappearance and reappearance of corneal reflex (corneal reflex) was registered. The results obtained were recorded and tabulated.

Statistical analysis: All the values were expressed as Mean \pm SEM. The differences were compared using one way Analysis of variance (ANOVA). The p values <0.05 were considered significant.

Table: 1 Duration of Surface Anesthesia in (Minutes) with Different doses of Alcoholic extract of *Terminalia chebula* (AETC) on Rabbit cornea

Groups n= 40	Pupil Size	Light Reflex	Loss of Corneal Reflex Mean \pm SEM	Reappearance of Corneal Reflex Mean \pm SEM	Duration of Surface Anesthesia Mean \pm SEM
Group I 2% Xylocaine	6mm	+	3 \pm 0.14	35 \pm 0	33 \pm 0.47
Group II 0.25% AETC	6mm	+	15 \pm 0.44	31 \pm 0	16 \pm 0.29**
Group III 0.5%AETC	6mm	+	10 \pm 0.33	33 \pm 0	23 \pm 0.44**
Group IV 1%AETC	6mm	+	05 \pm 0.14	46 \pm 0	41 \pm 0.25**

AETC- Alcoholic extract of *Terminalia chebula*, n-Number of animals, ** - p<0.000.

RESULTS

The time of disappearance of corneal reflex and the time between the disappearance and reappearance of corneal reflex were recorded in 40 albino rabbits after the administration of standard 2% xylocaine and three different doses of alcoholic extract of fruit of *Terminalia chebula*. At baseline (0 min), all the rabbits in four groups (n = 40) showed the presence of a normal corneal reflex, light reflex and the size of the pupil being normal. Light reflex and size of the pupil remained normal in all groups till the end of the experiment. Group I animals which

were delivered one drop of 2% Xylocaine had loss of corneal reflex at 3 minutes. Group II, Group III and Group IV which were delivered one drop of 0.25% AETC, 0.5%AETC and 1%AETC showed loss of corneal reflex at 15, 10 and 05 minutes respectively. Reappearance of corneal reflex has been observed at 35, 31, and 33 and at 46 minutes with 2% Xylocaine, 0.25% AETC, 0.5% AETC and 1% AETC respectively. Duration of surface anesthesia with 0.25% AETC, 0.5%AETC and 1%AETC was 16 \pm 0.29, 23 \pm 0.44 and 41 \pm 0.25 which was highly significant when compared with standard 2% Xylocaine (33 \pm 0.47) as shown in Table 1

DISCUSSION

Terminalia chebula is a species of *Terminalia*, native to southern Asia from India and Nepal east to southwestern China (Yunnan) and south to Sri Lanka, Malaysia and Vietnam [6,7]. It is used extensively in the Ayurvedic Medicine and in the Traditional Tibetan medicine. It is reputed to cure blindness and it is believed to inhibit the growth of malignant tumors [8]. The fruit of *T. chebula* has been extensively used in Thai traditional medicine for laxative, carminative, astringent, expectorant, and tonic effects [9]. The World Health Organization reported that 80% of the world population relies chiefly on traditional medicines involving the use of plant extracts or their active constituents [10]. Traditional healing system around the world utilizes herbal remedies and they are an important resource for the discovery of modern drugs [11].

The fruit of the tree possesses diverse health benefits and has been used as traditional medicine for household remedy against various human ailments since antiquity [3, 4, and Fruits are glabrous, ellipsoid to ovoid drupes, yellow to orange brown in colour. Found in deciduous forests of Indian sub continent, dry slopes up to 900 meters in elevation [4]. The plant has been extensively used in Ayurveda and Siddha for Constipation, diarrhea, ulcers, gastroenteritis, asthma, cough, dyspnea, dyspepsia, hemorrhoids, candidiasis, parasites, malabsorption syndrome, hepatomegaly, vesicular and renal calculi, urinary discharges, tumors, skin diseases, leprosy, intermittent fever, rheumatism, arthritis, gout, neuropathy, paralysis, memory loss, epilepsy, depression, diabetes, cardiovascular diseases, anorexia, wounds [12, 13]. *T. Chebula* possesses a wide variety of activities like antimicrobial [14], antioxidant [15], antiviral [16], anticarcinogenic [17], Hypocholesterolemic [18], Radio protective [19], antispasmodic & antipurgative [20]. *T. chebula* contain tannins which are of pyrogallol (hydrolysable) type. No of hydrolysable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-b-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terchebulin) have been isolated from fruits of *T. chebula* Phytochemicals like anthraquinones, ethaedioic acid, sennoside, 4,2,4 chebulyl-d-glucopyranose, terpinenes and terpinenols have also been reported to be present [21]. Triterpenoids and their glycosides have been isolated from stem bark of *T. chebula* [22]. Recent studies show that *T. chebula* contains more phenolics than any other plant [23, 24].

The paste of fruit is also applied in conjunctivitis for relief due to its anti-inflammatory property. The gargles of fruit decoction give excellent results in stomatitis and problems of the throat. A fine powder of fruit of *T. chebula* is used as a tooth powder to strengthen the gums. Aqueous extract of *T. chebula*, is used as a mouth rinse is used in caries of the tooth [25]. It is used in dysuria and urinary stones [26]. In one study the results of a 14-day orally repeated dose showed that *T. chebula* extract had no adverse effects at 2000 mg/kg body weight in rats [27].

As it relieves pain in conjunctivitis, stomatitis and problems of the throat, assuming that it might have local anesthetic action we conducted the above study. The result of the study shows that there is dose dependent local anesthetic action with three different concentrations of AETC on rabbit cornea as shown in Table 1. The rabbits were observed for ten days. There were no adverse effects like corneal abrasion, conjunctival injection etc. As there have been no developments of new molecules as local anesthetic agents for the last four to five decades, the fruit of *Terminalia chebula* can be taken for extensive research for the development of the same.

CONCLUSION

As the plant and parts of *Terminalia chebula* are used traditionally, for various health problems, further investigation and research is needed for the identification of biological activity of its phytoconstituents for development of an effective, safe and cost effective herbal drug for ophthalmic formulations which can be used as local anesthetic, for pain relief in conjunctivitis, stomatitis and problems of the throat as they have higher safety margins with minimum or no side effects.

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