Research Paper Pharma

Local Anesthetic Action of Aqueous Extract of Citrullous Colocynthis Fruit on Rabbit Cornea

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ABSTRACT

Local anesthetics are the drugs which cause reversible loss of sensations when applied locally. Citrullous colocynthis Schrad. from the family Cucurbitaceae, popularly named bitter apple. This study is to evaluate the local anesthetic activity of pulp of the fresh Citrullous colocynthis fruit. Aqueous extraction of fruit of Citrullous colocynthis (AECC) done by Soxhelts apparatus. Twenty male Rabbit's were taken and divided in to four groups each group contain 5 animals, Group I - Standard (2% Xylocaine), Group II - Test 0.25% Citrullous colocynthis extract. Group III - Test 0.5% Citrullous colocynthis extract. Group IV - Test 1% Citrullous colocynthis 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 Citrullous colocynthis

KEYWORDS Citrullous colocynthis, Corneal reflex, Light reflex, Local anesthetic

Introduction

Citrullous Colocynthis (Bitter apple) is a wild herb grows in the desert areas of Sindh and Punjab province of Pakistan [1]. In folk medicine, C. colocynthis is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent [2]. Citrullous colocynthis also shares certain analgesic and anti-inflammatory properties observed in *Citrullous lanatus* which belongs to same specie [3]. *Citrullous lanatus, Citrullous colocynthis, Citrullous vulgaris* are all from the Cucurbitaceae family. In the past some of the plant extracts were used as local anesthetics. The medicinal properties of *Citrullous colocynthis* are well known. Studies on *Citrullous colocynthis* anti-inflammatory and analgesic activities [4] suggest that *Citrullous colocynthis Schrad* (Cucurbitaceae), an herb which is endemic of Tunisia, can be used to treat certain inflammation disorders. The medicinal properties of *Citrullous colocynthis* used to tread Androgenic alopecia [5]. The dried pulp and seed extract of C. colocynthis is used for the treatment of constipation and diabetes traditionly. Other uses of *Citrullous colocynthis* include its use in folk medicine against dermatological, gynecological and pulmonary infections [6].Aqueous extract of leaves of *Citrullous Colocynthis* has local anesthetic action.[7] When you taste the pulp of the fresh fruit it causes numbness and tingling sensation. This led us to evaluate the local anesthetic property of the pulp of the fresh fruit of *Citrullous colocynthis*.

Material and Methods Plant Collection: The fruits of *Citrullous colocynthis* were collected from an agricultural field in month of May 2013, and authenticated in department of Botany at Government Degree College. Khammam.

Preparation of extract: The fruits of *Citrullous colocynthis* were shade dried and reduced to coarse powder in a mechanical grinder. The aqueous extract of the powdered material was obtained using soxhelts extractor. The extract obtained was evaporated at 45°C to get a semisolid mass. Phytochemical analysis of the extract was done. The study was performed between the months of July and August 2013, in the department of pharmacology, Mamata Medical College (MMC), Khammam, Andhra Pradesh.

Animals used: Twenty 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°C ± 2°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 Astra Zeneca, Alcoholic extract of *fruit of Citrullous colocynthis.*

Preparation of doses of aqueous extract of *Citrullous colocynthis* (AECC): 0.25% AECC, 0.5%AECC and 1%AECC prepared from the stock solution. Commercially available2% 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 initiat-

ed 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% AECC, 0.5%AECC and 1%AECC 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. 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±SEM. The differences were compared using one way Analysis of variance (ANOVA). The p values <0.05 were considered significant.

Results

The time of disappearance of corneal reflex and the time between the disappearance and reappearance of corneal reflex were recorded in 20 albino rabbits after the administration of standard 2% xylocaine and three different doses of alcoholic extract of fruit of *Citrullous colocynthis*. At baseline (0 min), all the rabbits in four groups (n = 20) 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 3.6 minutes. Group II, Group III and Group IV which were delivered one drop of 0.25% AECC, 0.5%AECC and 1%AECC showed loss of corneal reflex at 20, 12 and 6.6 minutes respectively. Reappearance of corneal reflex has been observed at 33 with 2% Xylocaine, and at 16, 23 and at 41 minutes, with 0.25% AECC, 0.5% AECC and 1% AECC respectively. Duration of surface anesthesia with 0.25% AECC, 0.5%AECC and 1%AECC was 16.13 ± 1.3, 23.4 ± 0.5and 41.1± 1 which was highly significant when compared with standard 2% Xylocaine (33.2± 0.98) as shown in Table 1

Table: 1
Duration of Surface Anesthesia in (Minutes) with Different doses of Alcoholic extract of *Citrullous colocynthis* (AECC) on Rabbit cornea

Groups n= 20	Pupil Size (mm)	Light Reflex	Loss of Corneal reflex (mint)	Reappearance of Corneal Reflex (mint)	Duration of Surface Anesthesia (mint)
Group I 2%Xylocaine	7	+	3.6 ± 0.67	36.1 ± 1.02	33.2± 0.98
Group II 0.25% AECC	7	+	20 ± 1.4	36.8 ± 1.02	16.13±1.3**
Group III 0.5% AECC	7	+	12 ± 0.7	34.8 ± 0.86	23.4 ± 0.5**
Group V1 1% AECC	7	+	6.6 ± 0.5	46.8 ± 0.58	41.1± 1**

AECC- Alcoholic extract of Citrullous colocynthis, n-Number of animals,
Values- Mean±SEM, **p<0.000

Discussion

Citrullous colocynthis, a member of the Cucurbitaceae family [8] is known as Bitter Apple in the English language [9], Hindal in Arabic [10] and Abujahl watermelon in Persian. The bitter taste and the powerful medicinal virtues of the pulp are due to the presence of a probably amorphous glucosid *colocynthin, first* identified and named by Meissner and by Vauquelin (1818), and later investigated and obtained more pure by Walz, Henke and others [11]. Cucurbitaceae, it is a climbing annual medicinal herb in the desert area distributed in African and Arabian countries and India. *Citrullous colocynthis* is a fruit commonly known as bitter cucumber, found in Sudan, Iran and India and in the deserts [12]. The fruits of *C.colocynthis* have been commonly used as antidiabetic medication in tropical and subtropical countries [13].This plant has insulinotropic effects [14], mild immunostimulating effects [15], antioxidant activity [16] and anticancer activity [17].Phytochemical screening revealed that *C. colocynthis* extract contains triterpene, flavonoids, tertiary and quaternary alkaloids, glycosides and saponin compounds [18]. One study reported a significant reduction of blood sugar level in diabetic rats that treated with aqueous extract of *C. colocynthis* seeds [19].Different parts of the plant including seeds, fruit, root, stem, and leaves, used as either aqueous or oil extracts, dried or fresh, are believed to have antidiabetic [14,20,21],antihyperlipidemic[22,23],laxative[20,21],anti-inflammatory[24], analgesic [24], vermifuge [25], hair-growth-promoting [26], antibacterial [27] , antifungal [27] , and antioxidant properties [1].The well known Phytochemicals of this plant are glycosides (Estrols classified to groups A, B, K, L, and E curcurbitacins), alkaloids, and flavonoids [28,29,30,31,17].The curcurbitacins (highly oxygenated tetracyclic glycosides) have a broad range of applications due to their wide spectrum of biological activities [1]. In high doses the plant is toxic, the adverse effects being bloody diarrhea and toxic colitis.The acceptable dosage of C. colocynthis fruit ranges 0.1–0.4 g/day according modern phototherapy. The seed should be administered at 120–300 mg (max: 600 mg) per day, and the root powder from 0.2 to 0.4 g/day. [36].The alkaloids of plant origin are used in medicine as an aesthetic agents [32].The milky latex from *Calotropis proceru* produces the nerve block anesthesia which contains alkaloid. [33].The anesthetic property of the extract is similar to that of xylocaine hydrochloride except for the time of duration. . Leaves of *Citrullous colocynthis* revealed nerve block anesthesia in one study. [7] As the local anesthetic action of leaves of *Citrullous colocynthis* known we conducted this study to evaluate local anesthetic action of fruit of *Citrullous colocynthis*. The result of the study shows that there is dose dependent local anesthetic action with three different concentrations of AECC 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. Local anesthetic action of fruit of *Citrullous colocynthis* may be due to presence of glycosides, alkaloids, and flavonoids. For the last four to five decades, there have been no developments of new molecules of local anesthetic agents. The fruit of *Citrullous colocynthis* can be extensively studied for the Phytochemicals responsible for local anesthetic action.

Conclusion

Different parts of the plant *Citrullous colocynthis*, including seeds, fruit, root, stem, and leaves are used traditionally, for various health problems. Research is going on for the identification of biological activity of its phytoconstituents for development of an effective, safe and cost effective herbal drug for various diseases. Alkaloids, glycosides, and other components of *C. colocynthis* pulp might be the cause of local anesthetic action which needs further investigation. Our study revealed local anesthetic property of the fruit of *Citrullous colocynthis*. A new molecule of local anesthetic activity like xylocaine can be developed as they have higher safety margins with minimum or no side effects.

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