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Intestinal Relaxant Activity of Crude Extract of Aesculus Indica and Its Underlying Mechanism

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ABSTRACT

Objective: To investigate the effects of *Aesculus indica* on rabbit's jejunum and its underlying mechanism. **Study Design:** Experimental study. **Settings:** Pharmacy Department, Bahauddin Zakria University, Multan, Pakistan. **Duration:** May 2017 to October 2017. **Methods:** The action of crude extract on rabbit's jejunum was examined. Each jejunal tissue of 1-1.5 cm in length was mounted in 15ml Tyrode's solution. The spasmolytic activity of crude extract of *Aesculus indica* was determined by treating each jejunal tissue with plant extract in doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10 µM cumulatively. Response was determined by percentage change in jejunal tissue contraction. To demonstrate the calcium channel blocking action of test plant, each jejunal preparation was first stabilized in Tyrode's solution having normal composition. To remove calcium from tissues, they were placed in calcium- free Tyrode's solution containing EDTA for thirty minutes. This solution was then replaced with potassium rich and calcium free solution. Verapamil (3µM) was used as a positive control. After incubation period of thirty minutes, control concentration-response curves of Ca^{++} (CaCl2) were achieved. **Results:** *Aesculus indica* produced spasmolytic effect on rabbit's jejunum in normal Tyrode's solution. Further evaluation of effect of *Aesculus indica* did not show the absolute inhibition of low K^+ induced contraction. To confirm whether the spasmolytic effect of *Aesculus indica* is through inhibition of Ca^{+2} channels or the opening of the K^+ channels, its effect was seen on the low K^+ (25mM) and high K^+ (80mM) induced contraction. *Aesculus indica* at the high dose did not show the absolute inhibition of low K^+ induced contraction. The crude extract of *Aesculus indica* showed concentration dependent relaxation of spontaneous, high K^+ (80mM) induced contraction with relevant EC_{50} values of 0.1083mg/ml (0.04793-0.2447, 95 %CI). **Conclusion:** The present study shows that *Aesculus indica* has spasmolytic effect which is due to Ca^+ channel blocking activity.

Keywords: *Aesculus indica, Verapamil, Tyrode's solution, Ca+ channel.*

INTRODUCTION

Calcium channels are of T, L, N, P, Q and R types. L-type Ca2+ channels are also known as the high voltage gated channels while the T-type Ca2+ channels are low voltage-gated. Upon depolarization L-type channels are slowly activated than T-type calcium channels but shows higher communication in smooth muscle cells. Calcium ions required for initiation of excitation and contraction is made available by these channels.[1] Cytosolic calcium is increased following calcium channel opening after membrane depolarization in smooth muscle cells. However, in gut especially in large and small intestines; periodic motor pattern is observed. There is an association where mediators and neurotransmitters when operated shows slow waves origination in interstitial cells of cajal. These cells functions as pacemaker cells by

controlling frequency of contractions. When calcium reaches the threshold, these interstitial cells being important component of GIT sends signals to smooth muscles and hence actin potential is generated.[2]

Half of the ion channels superfamily is made up of potassium (K^+) and voltage-sensitive potassium (K_V) channels are the largest group. The wide variety of K^+ channels detected in ICCs and SMCs of the GIT reflects their rich diversity. When the intracellular K^+ concentration becomes 10-fold higher than extracellular, K^+ channel open and produces an outward K^+ flux that turns the membrane potential in the negative direction toward K^+ reversal potential. As a result, K^+ channels are responsible for the maintenance of resting potential, plateau current and repolarization of slow waves. Many signaling pathways target these channels.[1]

Worldwide, ethno medicinal uses of plants have been testified.[3,4] Use of medicinal plants by humans can be traced approximately 5000 years back, yet in modern era plants are one of the vital sources of recent remedies such as tubocurarine, ephedrine, quinine, aspirin and digoxin.[5,6,7]

Since, allopathic medicines are associated with more adverse effects and difficult to approach health units particularly in rural areas therefore, even in this modern progressive era where allopathic goes parallel to herbology; the local man's reliance on plants and herbs for their ailments have gained massive popularity, endorsing fewer number of side effects and budget friendly.[8,9]

*Aesculus indica (*Ai. Cr*)* is a medicinal plant which is spread mainly in the colder region all over the world and belongs to the *Hippocastanacea* family. It is usually known as Bankhor, Kanor, Kanor, Pangar, Himayalan chest nut or Indian horse chest nut. This plant has been a great focus of research for centuries due to its medicinal properties. Seeds, bark and roots are used for rheumatism; fruits are used as anti-diabetic and in colic disorder; leaves possess anti-cancer properties. It is highly useful in hemorrhoids, varicose veins and ulcers to prevent thrombosis. It often aids in the treatment of migraine, blood effusions and frost bite.[10]

Various prokinetic agents (which increases gut motility) are already in use for these conditions but they are associated with a lot of adverse effects e.g., anticholinergic drugs give good prokinetic effect but they also have multiple muscarinic as well as nicotinic side effects, decreased heart rate, hypotension or even cardiac arrest, flushing and precipitation of bronchial asthma attack may also occur. Metoclopramide and Domperidone, dopamine antagonists, are also used to enhance gut motility but are associated with extra pyramidal manifestations such as neuroleptic syndrome and tardive dyskinesia, prolongation of the corrected QT interval, sedation and diarrhea.[11]

The present study was conducted to investigate *Aesculus indica* effect on the intestinal motility and to explore its underlying mechanism. The results of the study will provide a good insight to the treatment of gastrointestinal (GI) disorders like diabetic gastroparesis, gastroesophageal reflux and other causes of gastrointestinal dysmotility.

METHODS

Drugs and other chemicals: Verapamil hydrochloride (Sigma Chemicals Co.) was purchased from local market. Chemicals required for preparing solution were magnesium chloride, ethylenediamine tetra acetic acid (Sigma), potassium dihydrogen phosphate, calcium chloride, sodium bicarbonate, magnesium sulphate, potassium chloride, glucose, sodium chloride, potassium dihydrogen phosphate and sodium dihydrogen phosphate (Merck).

Animals and data recording: Twenty-eight rabbits, weighing 200-220g were used in this study. These were kept in the animal house of Bahauddin Zakria University, Multan. They were provided with water *ad libitum* and diet containing cabbage, carrots, spinach, and cauliflower. They were kept overnight fasted earlier the experiment and were given access to water only. Temperature was kept at 25±1°C. At the day of the experiment, they were sacrificed. Animals found either pregnant or in diseased state were excluded from the research. Humane care was given to animals according to the criteria defined in "guide for the care and use of laboratory animals".[12,13] They were kept for acclimatization for one week.[14,15]

Method of preparing crude extract of *Aesculus indica*: Plant substance rendered free from soil and polluted material, and shade dried and coarsely powdered. Material saturated into ethanol (70%) with 7 days intermittent shaking. Passed throughout a muslin stuff and liquid portion passed through a sift piece (Williamson *et al*., 1998). Filtrate was evaporated under reduce pressure (760*mmHg*), semisolid paste of dark green, that was transferred to petri dish and positioned at room temperature to evaporate the left after solvent. The above-mentioned extraction procedure was repeated two times to obtain greatest yield. The crude extract was transferred to a glass bottle and stored in refrigerator (-4 ^0C) until used.

Tissue preparation: (*Rabbit's Jejunum)* The action of crude extract on rabbit's jejunum was examined. Each jejunal tissue of 1-1.5cm in length was mounted in 15ml Tyrode's solution, whose composition in mM was: NaCl

8, $MgCl_2$ 0.1, KCl 0.2, $NaHCO_3$, $CaCl_2$ 0.2, NaH_2PO_4 0.05, Glucose 1 gm/liter.[16]

Data Collection Procedure: All tissues after suspending in relevant solution were kept at 37 °C and supplied by 5% CO_2 and 95% O_2. Each tissue was permitted to get stabilize for 30min prior to addition of plant extract. 1gm tension was applied to every tissue and kept constant throughout the experiment. Response of each jejunal tissue was recorded for 2min by using Bioscience transducers connected to a power lab (AD instrument, Sydney, Australia) and computer system.

Spasmolytic Activity: The spasmolytic activity of *Aesculus indica* was determined by treating each jejunal tissue with gradually increasing doses of plant's crude extract in doses. Doses were as 0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10 µM and cumulative dose responses curves were constructed for each tissue. Cumulative dose response curves were compared for percentage change in each jejunal tissue for height of contraction. Verapamil (3µM) was used as a positive control.

To investigate the spasmolytic effect of *Aesculus indica* that whether it is through inhibition of Ca^{++} channels or opening of K^+ channels, its effect was considered on low K^+ (25mM) and high K^+ (80mM) induced contraction. Jejunal preparations were depolarized with high K^+ (80 mM), as KCl, persistent contractions were produced when K^+ (80 mM) was added to the tissue bath. The plant extract was then added without washing the tissue to get dose-dependent inhibitory response.

To demonstrate the calcium channel blocking action of test plant, each jejunal preparation was first stabilized in Tyrode's solution having normal composition. To remove calcium from tissues, they were placed in calcium- free Tyrode's solution containing EDTA for thirty minutes. This solution was then replaced with potassium rich and calcium free solution. Verapamil (3µM) was used as a positive control. After incubation period of thirty minutes, control concentration-response curves of Ca^{++} (CaCl2) were achieved. After two cycles the control CRCs of Ca^{++} were found super imposable and tissues were pretreated for sixty minutes with crude extract of *Aesculus indica* to check the blockade of Ca^{++} channels. The CRCs of Ca^{++} in the presence of various concentrations of *Aesculus indica* were reconstructed.

Statistical Analysis: Percent response (%) of each concentration of test drug alone and in the presence of respective verapamil was plotted as dose response curve in Graph pad prism 8.0.2. *t*-test was used to compare the responses. *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

Effect on *Aesculus indica* on Rabbit Jejunum: The crude extract of *Aesculus indica* was considered for its spasmolytic effect on the spontaneous contractions of isolated rabbits' jejunal preparations. It showed dose dependent inhibition of the contraction of jejunal tissues.

In order to investigate the involvement of calcium and potassium channels in producing spasmolytic effects, the effects of *Aesculus indica* was evaluated on both low (25mM) and high K^+ (80mM) as well as in the presence of verapamil (calcium channel blocker).

Aesculus indica crude extract produced dose dependent inhibitory effect on jejunal contractions in concentration–response curve of Ca^{++} that is comparable to verapamil induced reduction in jejunal contractions in ca++ treated tissues. Moreover, *Aesculus indica* at high dose did not show the absolute blockage contraction induced by low K^+. The crude extract of *Aesculus indica* showed concentration dependent decrease in spontaneous, high K^+ (80mM) induced contraction with relevant EC_{50} values of 0.1083mg/ml (0.04793- 0.2447, 95 %CI).

Figure 1: Calcium response curves of *Aesculus indica*

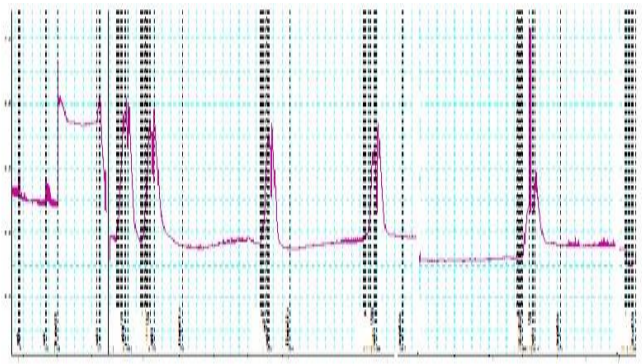


Figure 2: Dose-dependent inhibitory effect of crude extract of *Aesculus indica* (Ai. Cr) and verapamil on spontaneous rabbits' jejunal preparations (Mean ± S.E. n = 7)

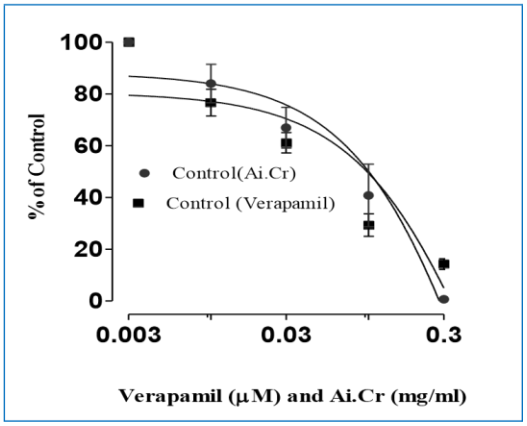


Figure 3: Dose-dependent inhibitory effect of crude extract of *Aesculus indica* and verapamil on high K^+ (80Mm) induced contraction in isolated rabbits' jejunal preparations (Mean ± S.E. n = 7) P-value < 0.05*

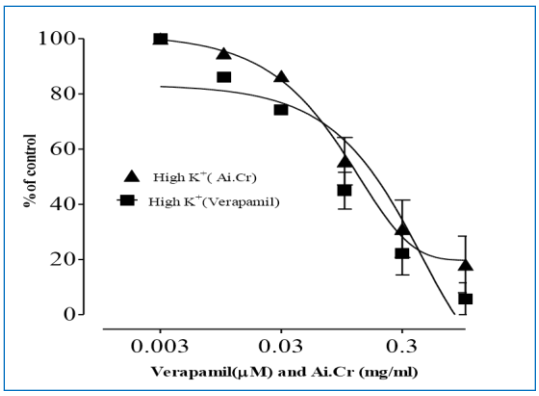


Figure 4: Dose-dependent inhibitory effect of crude extract of *Aesculus indica* on spontaneous and high K^+ (80Mm) induced contraction in isolated rabbits' jejunal preparations (Mean ± S.E. n = 7) P-value < 0.05*

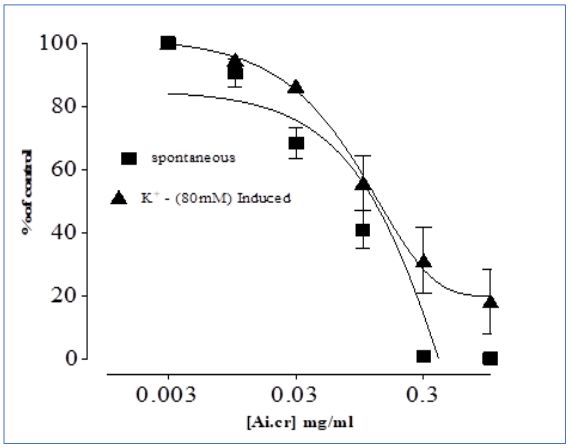


Figure 5: Effect of verapamil on spontaneous and high K^+ (80 mM) - induced contractions in isolated rabbits' jejunal preparations (Mean ± S.E. n = 7)

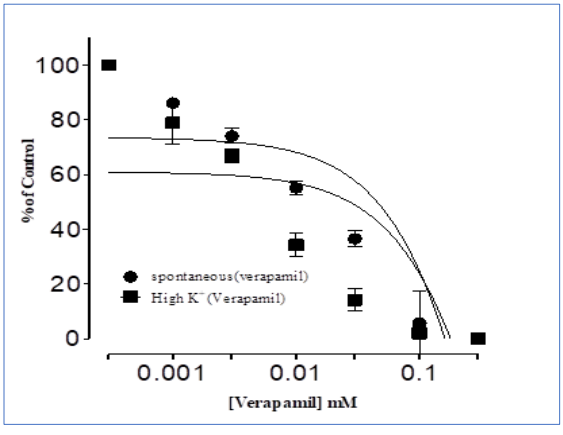


Figure 6: Concentration –response curve of Ca^+ in presence of *Aesculus indica.* (Mean ± S.E. n = 7)

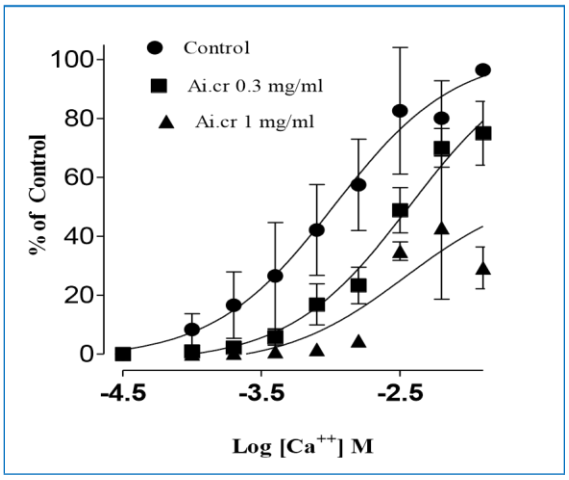
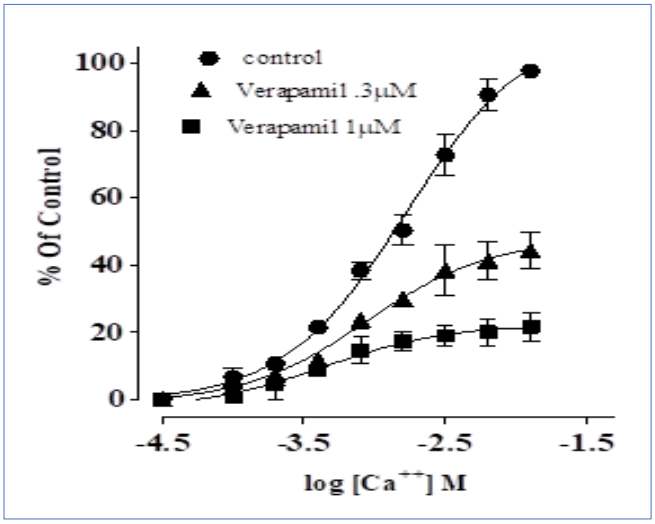


Figure 7: Concentration–response curve of Ca^+ in presence of Verapamil



DISCUSSION

The crude extract of *Aesculus indica* caused significant decrease in spontaneous and high K^+ induced contraction in isolated preparation of rabbits' jejunum. For investigating spasmolytic activity of *Aesculus indica*, calcium response curves were constructed, which confirmed the association of spasmolytic activity with the blockade of calcium channels.

Previous experiments performed to determine spasmolytic activity of medicinal plants revealed many underlying mechanisms but for majority the blockage of Ca^{++} channels was found to have significant role.[17] To evaluate the possible method of antispasmodic effect, *Aesculus indica* has been tested on high K^+ (80 mM) and low K^+ Induce contraction. Concentration of K^+ if exceeds than 30mM, it is capable of inducing smooth muscle contraction by opening of L- type voltage-dependent Ca^{++} channels, thus permitting the influx of extracellular

calcium to produce contraction.[18] Following maximum effect of Potassium, *Aesculus indica* was added in a cumulative mode that caused a dose-dependent relaxation. It may be due to limited Ca^{++} entry via voltage gated channels. Pre-treatment of jejunal tissues with *Aesculus indica* made shift to right in the Ca^{++} curves at a dose of 0.1-0.3mg/ml like verapamil (Ca^{++} channel blocker) which made changes in Ca^{++} curves at doses of 0.03 - 0.1µM. The noticed effects of *Aesculus indica* inhibited contractions induced by K^{+} which were followed by displacing effects of high Ca^{++} concentrations, it suggests the existence of a Ca^{++} channel blocking action of *Aesculus indica*.

Increase in intracellular calcium causes gastrointestinal smooth muscle to contract. Normally, Ca^{++} influx is triggered by membrane depolarization and this Ca^{++} works as stimulator for contraction. Various studies have shown that GI smooth muscles loses spontaneous contractions when exposed to calcium-free solution.[19] In smooth muscle cell cytoplasm, binding of Ca^{++} to calmodulin occurs, calcium-calmodulin complex combines with myosin light chain (MLC) kinase. It results in the formation of a receptor which leads to phosphorylation of serine at position 19 in the myosin light chain, this in turn allows myosin ATPase to be activated by actin and muscle contraction takes place.[20]

CONCLUSION

The present study shows that *Aesculus indica* has spasmolytic effect which is due to Ca^{++} channel blocking activity. This provides a therapeutic source for its usefulness in gastrointestinal diseases.

LIMITATIONS

As this study is based on single center, therefore more such studies are required to generalize the results. Moreover, as the study is performed in controlled environment, so there are chances of human error.

SUGGESTIONS / RECOMMENDATIONS

Further pre-clinical trials shall be conducted to compare its efficacy with already available anti-spasmodics in the market.

CONFLICT OF INTEREST / DISCLOSURE

The authors declare no conflict of interest.

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