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ACUTE TOXICITY AND RELAXANT ACTIVITY OF TOTAL FLAVONOIDS OF FORSSKAOLEA TENACISSIMA.

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ABSTRACT

BACKGROUND: Abdominal spasms have historically been treated with the whole plant of *Forsskaolea tenacissima*. Thus, the study's goals were to identify the safe dosage range, identify the mechanism or mechanisms behind *Forsskaolea tenacissima's* therapeutic usage for gastrointestinal spasms, and extract the plant's total flavonoids. **METHODS:** *Forsskaolea tenacissima's* total flavonoids were examined for potential antispasmodic efficacy in isolated rabbit jejunal preparations after an acute toxicity research was conducted to establish the safe dosage range prior to in vivo tests. **RESULTS:** With comparable EC50 values of 4.22 ± 0.849 mg/ml and 0.607 ± 0.0306 mg/ml, total flavonoids from *Forsskaolea tenacissima* decreased both spontaneous and high K+-induced contractions in isolated rabbit jejunal preparations. The concentrations used were 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, and 10.0 mg/ml. The mechanism was verified by constructing a calcium curve in the decalcified rabbit jejunal preparation with and without verapamil 0.1, 0.3 µm. The EC50 value of the total flavonoids in *Forsskaolea tenacissima* is -2.55 ± 0.00 at 0.1 mg/ml, whereas the control has an EC50 value of -2.80 ± 0.00. In the presence and absence of 0.1 µM verapamil, the corresponding EC50 Log Ca++ M values are 1.71 ± 0.07 and -2.45 ± 0.00, respectively. This suggests that the total flavonoids of *Forsskaolea tenacissima* follow voltage-gated calcium channels for calcium influx since the right shift of Verapamil and the test sample's total flavonoids right shifts are comparable. **CONCLUSION:** According to the study, *Forsskaolea tenacissima's* total flavonoids have antispasmodic action that may be mediated via voltage-gated Ca++ channel blockage, and the safe dose is 100 mg/kg. This gives the plant a solid pharmacological foundation for its potential medical application in treating intestinal spasm.

KEYWORDS: *Forsskaolea tenacissima*, Flavonoids, Antispasmodic, Ca++ antagonist, Verapamil

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INTRODUCTION

From 16th to 18th century human beings were using medicinal plants in the form of maceration, decoction and infusions and these medicinal plants were in the form of compounded drugs which were of plant and animal origin. In the 19th century Ipecacuanha, quinine, pomegranate, glycosides, saponosides, vitamins, hormones, tannins were isolated from plants and during the 20th century the concept that the pharmaceutical products are only obtained from plant source has changed and many pharmaceutical products were prepared from synthetic source [1, 2].

Forsskaloea was named in mourning of a student Peter Forsskal Swedish Botanist who died while collecting zoological and botanical specimens from the Arabia Felix [3]. Forsskaolea is a small genus of about 6 species distributed in Algeria, Egypt, Spain, Malta, Israel, Jordan, Libya, Palestine, Sinai, Tunisia, Saudi Arabia, Oman, United Arab Emirates, Afghanistan, Iran, India and Pakistan [4-6]. *Forsskaolea tenacissima* belongs to Urticaceae family [7, 8] which has 48 genera and 1050 species distributed throughout the world, mostly in tropical regions while in Pakistan it has six genera and nine species [9]. Its flowering season lasts from March to June, and it is mostly found in regions such as North Africa, Saudi Arabia, Palestine, Afghanistan, Iran, India, Pakistan, and South West Europe. [10, 11]. Flavonoids are polyphenolic compounds which are present in fruits, seeds, bark, nuts, vegetables, wine, flowers, honey, tea, etc. and are present in cells of plants as well as in human supply [12] . The US consumes 500–1000 mg of mixed flavonoids each day [13]. Flavonoids have different classes. These include flavones, chalcones, flavonoid, isoflavones, catechins or flavanols, dihydroflavonols, anthocyanidins and flavanones [14]. Flavonoids have anti-inflammatory, antibacterial, anti-allergic, vasodilatory activities and antiviral. Flavonoids serve a variety of purposes. They give flowers their color, and they assist in the physiological survival of plants by shielding them from UV-B rays and fungi. Additionally, flavonoids regulate respiration,
morphogenesis, photosynthesis, sex determination, and energy transmission [15-18]. Flavonoids have different adverse effects like acute renal failure, haemolytic anemia, fever, hepatitis, thrombocytopenia, skin reactions are caused due to drug such as cianidanol when its dose is increased from 1 to 1.5 mg/day [19-21] also tea which is a rich source of flavonoids but black tea has hydrolysable tannins tannic acid cause inhibition of iron absorption. Phenolic monomers, polyphenols, tannins, which are phenolic compounds form insoluble complexes in GIT lumen due to which iron bioavailability is reduced. Galloyl groups, but not catechol groups, have been linked to the phenolic compounds' in vivo suppression of iron absorption [22, 23]

MATERIALS AND METHODS

PLANT MATERIALS

The whole Forsskaolea tenacissima plant was used to assess the plant's acute toxicity and spasmolytic activity. The plant was obtained from the Institute of Basic Medical Sciences at Khyber Medical University, where Professor Dr..... identified it. A voucher specimen of the plant was also sent to the department of

pharmacology at IBMS, KMU, Khyber Pakhtunkhwa, Pakistan.

Drugs and standards Chemicals of analytical quality were employed in the bioassay procedures. Acetylcholine was acquired from NorthWest General Hospital & Research Centre, Hayatabad, Peshawar, and utilized for tissue maintenance at quiescent dosages. All of the solutions were made fresh on the day of the studies.

Animals Locally, both sexes of rabbits were breaded. The Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan's ASRB and Ethical Committee report that they were housed at the "Animal House of Institute of Basic Medical Sciences, Khyber Medical University," weighing between 2.0 and 2.5 kg on average.

Preliminary Phytochemical screenings To verify the presence of flavonoids, a preliminary phytochemical analysis of the methanolic extract of *Forsskaolea tenacissima* was conducted using the Alkaline Reagent Test and the Lead Acetate Test. The Alkaline Reagent Test called for mixing 10 milliliters of distilled water with one gram of dried and powdered *Forsskaolea tenacissima*, then boiling the mixture for five minutes. After the filtrate was obtained hot, a few drops of sodium hydroxide were added after it had cooled to room temperature. After appearing and then disappearing when a few drops of acetic acid were added, the existence of flavonoids was verified [24]. For Lead Acetate Test, in a test tube containing 1 ml plant methanolic extract, 1 ml of 10 % lead acetate was added. The mixture was let to stand for a while without being touched. The presence of flavonoids was verified by the precipitate formation [25].

Extraction of plant materials Plant 10 kg was subjected to washing with distill water, shade drying and grinding into fine powder weighing 4.8 kg. Commercial-grade methanol 80% was used to macerate the plant for a week. Using regular filter paper, the mentrum was filtered. The filtrate produced by a rotary evaporator at 50°C was a semisolid, dark greenish extract devoid of methanol. Some of this extract was set aside for use in pharmacological testing. The remaining extract was suspended in distillation water and then fractionated using n-hexane and ethyl acetate in turn.

Fractionation of total flavonoids of *Forsskaolea tenacissima*

In a separating funnel, 100 milliliters of distillation water were used to dissolve the dried methanolic extract, and then 100 milliliters of n-hexane were added. The mixture was agitated for a time before being put aside to allow the components to separate between the aqueous and n-hexane layers. A pipette was used to transfer the n-hexane portion into a beaker, where it was disposed of in order to separate the organic and aqueous layers. After that, the aqueous component was poured into an equivalent volume of ethyl acetate and agitated gently in a funnel for a while before being permitted to stand still. After being moved into a beaker, the top layer of ethyl acetate was allowed to concentrate in a rotary evaporator set at 180 revolutions per minute rpm at 50 degrees Celsius with lowered pressure. Total flavonoids were abundant in the concentrated dark greenish residue that was produced by the phytochemical test i.e., TLC 26.

Acute toxicity

Six groups of 30 mice, one of each sex, were created. Each group contained four mice. Intraperitoneal injections of *Forsskaolea tenacissima* total flavonoids at test doses of 1, 10, 100, 1000, 2000, and 3000 mg/kg were administered to mice in each group. The animals used in the experiment were under constant observation for twenty-four hours. LD50 was computed after 24 hours, and the number of deaths in each group was recorded.

Spasmolytic activity

Total flavonoids of *Forsskaolea tenacissima* was screened for possible spasmolytic activity. Rabbits were slaughtered and their abdomens were opened. The jejunum portions of the rabbit, which were mounted in a tissue bath with 15 ml of Tyrode's solution at 37 °C and continuously supplied with Carbogen gas 95 % oxygen and 5 % carbon dioxide, were around 2 to 2.2 cm long. NaCl 136.9, KCl 2.68, CaCl2 1.8, NaH2PO4 0.42, MgCl2 1.05, NaHCO3 11.90, and glucose 5.55 were the concentrations utilized to create Tyrode's solution. After allowing the tissues to acclimate to Tyrode's solution for half an hour, they were calmed for at least five minutes with sub-maximal acetylcholine concentrations 45 microliters of 10-4 M while waiting for consistent responses. Once the tissue was stabilized, total flavonoids of *forsskaolea tenacissima* were tested in cumulative manner and results were recorded. The test doses of the flavonoids were 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, 10.0 mg/ml 27 The tissue was then depolarized and the jejunum was made to contract using high 80 mM KCl. The jejunum was relaxed with total flavonoids at the same doses, and the percentage of relaxation response to contractions caused by KCl was noted 28. In order to examine the mechanism of action, calcium chloride curves were developed. After stabilizing the tissue it was decalcified by using K – normal solution and K –rich solutions

Tyrode's normal was used to stabilize the tissues for 30 to 40 minutes. After that, the tissues were decalcified by being washed five times with K-rich solutions and twice with K-normal solutions. Using calcium chloride as a control, Ca++ curves were constructed at concentrations ranging from 1 to 256 ×10-4 M. After that, Tyrode solution was used to cleanse the tissues once again. After decalcifying the tissues once again as previously mentioned, extracts with varying concentrations 1–15 mg/ml were employed. Curves were created after an hour of incubation and the addition of calcium chloride. After the EC50 values were determined, they were compared to the appropriate control. Likewise, verapamil 0.03–0.1 mg/ml and its absence were used to generate concentration response curves. They were found to have EC50 values. Curves were then compared for potential shifts to the right.

Data recording and Interpretation

Intestinal recordings were made using an isotonic transducer MLT 0210/A Pan Lab that was coupled to a Power lab Model No: 4/25 T. Bridge between Australia and AD instruments The intestinal signal was amplified using a pod amplifier that was linked to the Power lab

Dose mg/kg body weight			
1st Phase	Group 1 1 mg/Kg	Group 2 10 mg/Kg	Group 3 100 mg/kg
	All Alive	All Alive	All Alive
2nd Phase	Group 4 1000 mg/kg	Group 5 2000 mg/kg	Group 6 3000 mg/kg
	One Died	Two Died	All Died

responses. Data interpretation was done using Lab Chart 7, which was utilized with the Power Lab.

Data analysis

Graph Pad Prism was used to provide the median effective concentrations EC50 values along with 95% confidence intervals CI, and the data are shown as mean ± standard error of the mean SEM.

Results and discussion 10 kg of *Forsskaolea tenacissima* yielded 50 gm of total flavonoids. In phase 1 animals of group 1, 2 and 3 were given test dose of 1 mg/kg, 10 mg/kg and 100 mg/kg respectively but it didn't show any lethality which means the lethality is zero percent while in second phase in group 4 one trial animal, in group 5 two trial animals and in group 6 all trial animals were killed which means that the lethality

was 25 %, 50 % and 100 % respectively in this phase.

Table 2 Findings of *Forsskaolea tenacissima's* total flavonoids' acute toxicity in mice

Figure 2 Acute toxicity test of *Forsskaolea tenacissima's* total flavonoids in a mouse model, graphically presented

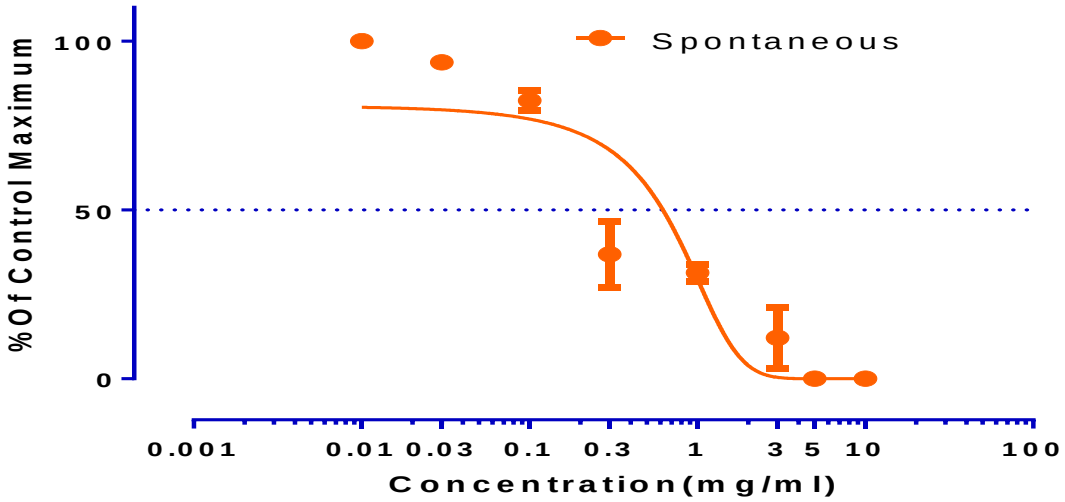
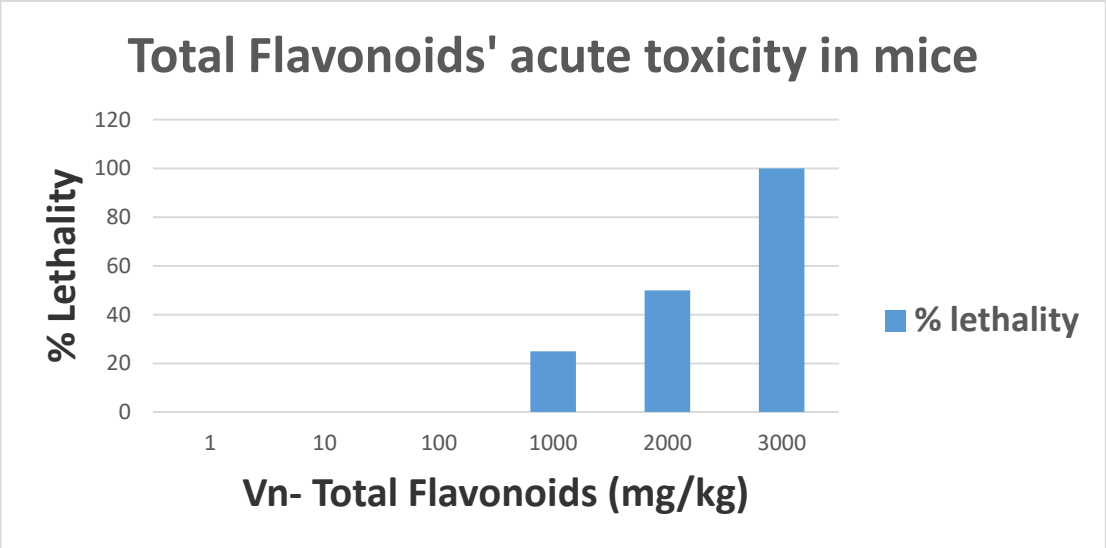


Figure 3A visual representation of the relaxing effect of total flavonoids on prolonged, spontaneous contractions



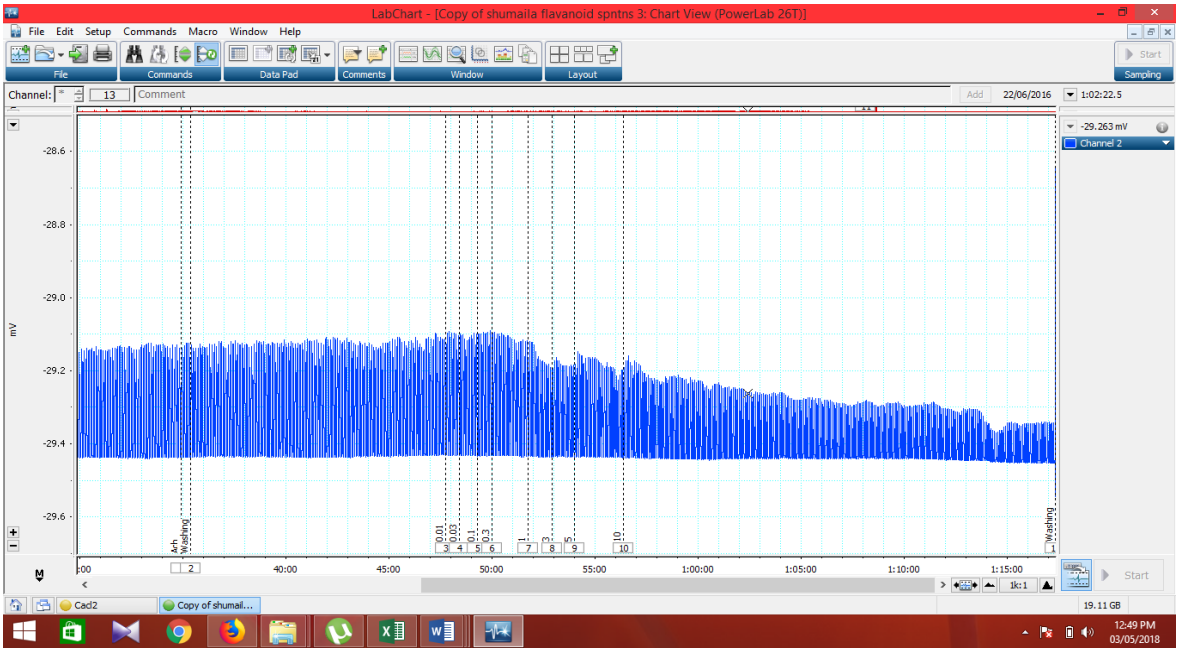


Figure 4 Showing the relaxing effect of *Forsskaolea tenacissima's* total flavonoids on spontaneous rabbit jejunal preparations via graph tracing Figure 3.3 Explain how *Forsskaolea tenacissima's* total flavonoids affect spontaneous jejunal preparations. The amplitude of spontaneous contractions decreases in concentration. Beginning at 0.03 mg/ml, the spasmolytic effect fully relaxes the spontaneous spasms at 3 mg/ml. The EC50 value for the spasmolytic effects of Forsskaolea tenacissima's total flavonoids on spontaneous spasms is 0.607 ± 0.0306

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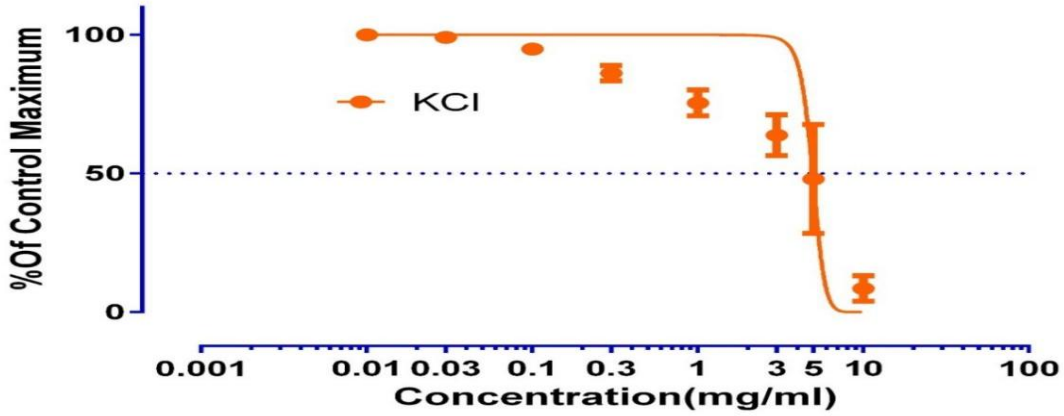

Figure 5 Visual representation of the relaxing effect of total flavonoids on prolonged contractions of the KCL

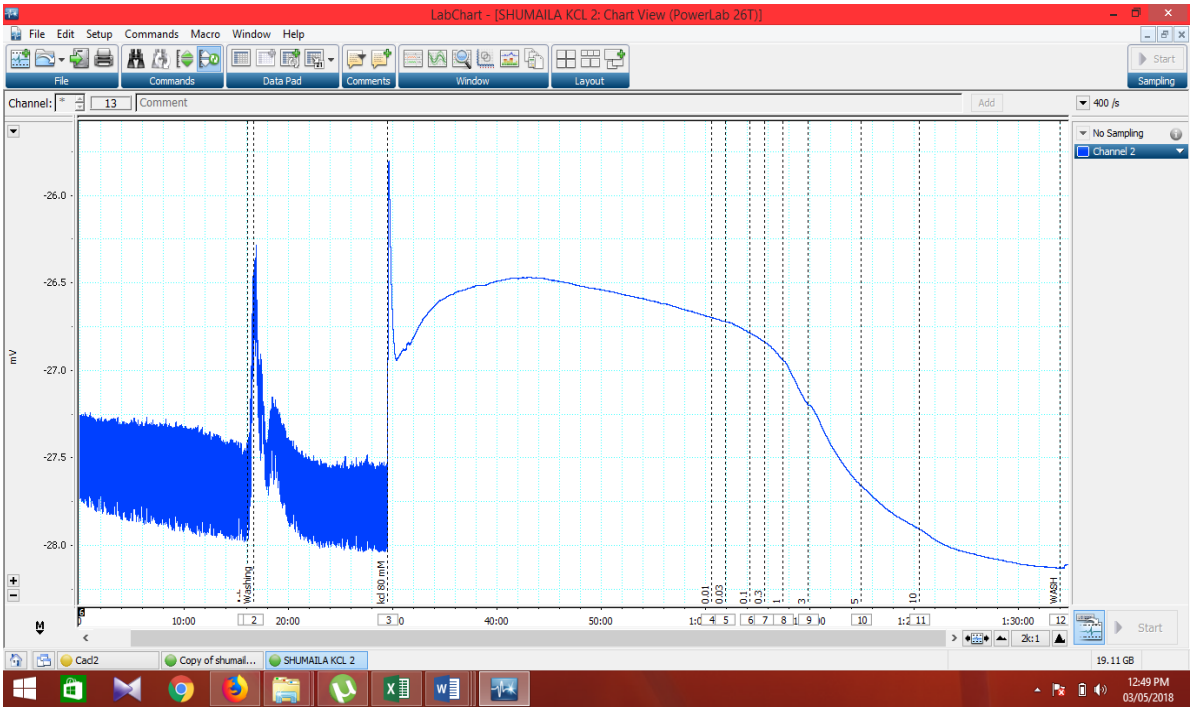

Figure 6 Graph tracing for the relaxing effects of total flavonoids on rabbit jejunal preparations' KCl-sustained contractions Figure 3.5 explain how the total flavonoids of Forsskaoleatenacissima affect the contractions that are generated in rabbit jejunal preparations by KCl 80 Mm. Its amplitude was reduced by various concentration dosages. At 0.03 mg/ml, a relaxant effect was seen, and at 10 mg/ml, the contraction was totally relaxed. For prolonged contractions in KCl, its EC50 values were 4.22 ± 0.849.

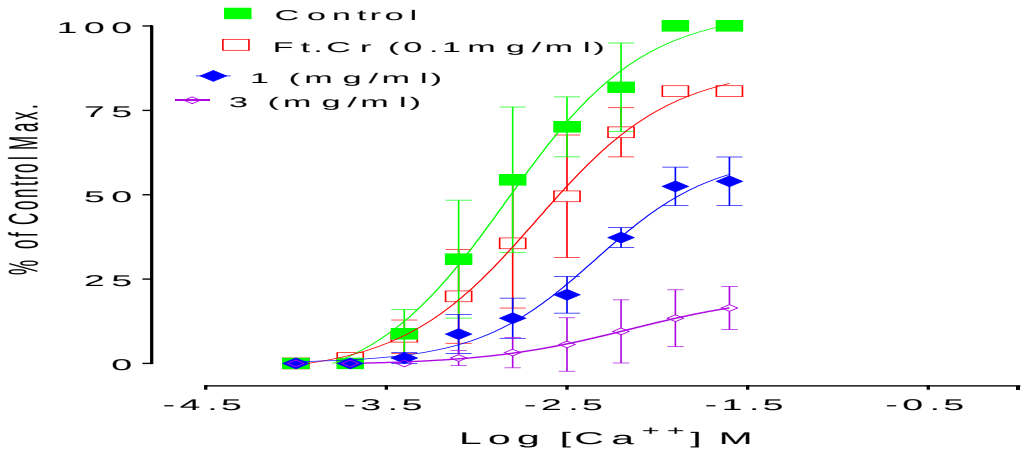

Figure 7 Graphical presentation of calcium chloride curves of total Flavonoids of *Forsskaolea tenacissima* in the presence and absence of verapamil

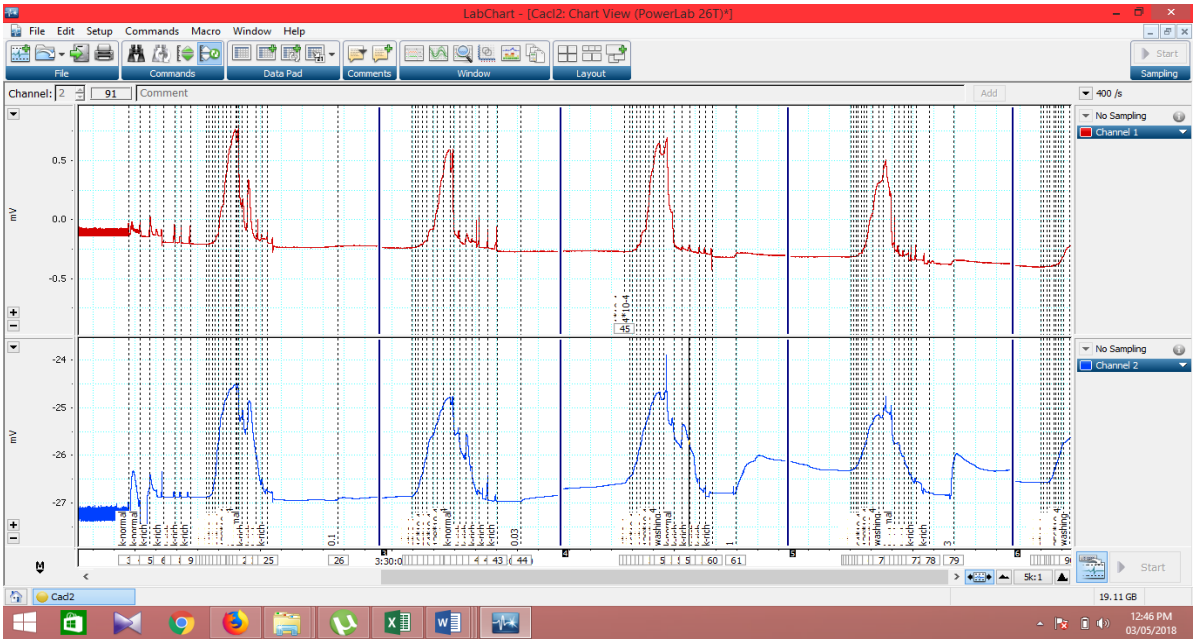

Figure 8 An illustration of how the total flavonoids of *Forsskaolea tenacissima* affect the calcium chloride control curve Figure 3.7 shows the calcium curve for the total flavonoids of the extract from *Forsskaolea tenacissima* reaches its greatest amplitude, or 82% of the control maximum, at 0.1 mg/ml, while it barely approaches 54% of the control maximum at 1 mg/ml. The fraction test sample had an EC50 value of -2.55 ± 0.00 at a concentration of 0.1 mg/ml, whereas the control had an EC50 value of -2.80 ± 0.00. This suggests a movement to the right in comparison to the control's EC50 log Ca++ M. Likewise, the corresponding EC50 Log Ca++ M when 0.1 µM verapamil is present is 1.71 ± 0.07 and -2.45 ± 0.00. By comparing the graphs of the test sample and verapamil, we can see that the latter's right shift is similar to the test sample's the ethyl acetate fraction right shift. This leads us to the conclusion that Forsskaolea tenacissima's ethyl acetate component influxes calcium by following voltage-gated calcium channels.

CONCLUSION

According to this study, *Forsskaolea tenacissima's* total flavonoids have antispasmodic properties through Ca++ channel blocking, which offers a solid pharmacological foundation for its application in treating intestinal spasms.

List of abbreviations:Ft.cr: Crude methanolic extract of *Forsskaolea tenacissima* **CRC's:** Concentration-response curves **CCB:** Calcium channel Blocking.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared

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