KENYATTA UNIVERSITY

SCHOOL OF HEALTH SCIENCES

CHOLINERGIC ACTIVITY OF ACTIVITY OF MMMETHANOLIC LEAF EXTRACT OF *PLECTRANTHUS BARBATUS* IN WISTER RATS

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BACHELOR OF PHARMACY

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A RESEARCH DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A BACHELOR OF PHARMACY DEGREE.

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SEPTEMBER 2023

DECLARATION

Student

I declare that this project is my original work and has not been presented at any other university.

Signed …… Date…….

Okong'o Ouma P110/2974/2018

Supervisor

This research dissertation has been submitted to the fulfillment for a Bachelor of Pharmacy degree award with my approval as the university supervisor.

Signed…… Date……...

Dr. Siambi Kikete

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

GIT	Gastrointestinal Tract
CNS	Central Nervous System
PNS	Peripheral Nervous System
HPLC	High Performance Liquid Chromatography
MIC	Minimum Inhibitory Concentration
BHI	Brain Heart Infusion
AChE	Acetylcholinesterase
ACh	Acetylcholine
KSh	Kenya Shillings
DMSO	Dimethyl sulfoxide

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ABSTRACT

Background: *Plectranthus barbatus*, a medicinal herb known for its potential therapeutic properties from the lamiaceae family, has gained attention for its possible role in modulating cholinesterase activity, which is implicated in various neurological and gastrointestinal disorders such as gastroparesis and constipation (Mbaria et al, 2023). This study assessed the cholinergic activity of *Plectranthus barbatus* extract within the gastrointestinal tract of Wister rats, shedding light on its potential therapeutic applications in the GIT as this would pave way for further research and development of safer and cheaper natural remedies for management of such conditions.

Objectives: To evaluate the effect of *Plectranthus barbatus* methanolic leaf extract on the gastrointestinal cholinergic activity. The dose response studies of the plant extract. To compare the contraction generated by the plant samples with that of acetylcholine.

Methods: In vitro experiments utilizing rat intestines was employed to investigate the impact of *Plectranthus barbatus* extract on cholinergic activity. The plant extract was prepared by maceration process, from which two concentrations were prepared. The intestines were preserved in an organ bath, where they were treated with the plant extract in different concentrations, and the positive control. The smooth muscles will be connected to an external transducer to record the contractions in form of waves on a graph paper.

Results and conclusion : The study results indicated that *P.barbatus* extract showed significant contraction on the intestine smooth muscles or Wister rats. Higher doses of the sample produced more contractions . The contractions were less than that of acetylcholine treated muscles. Onset and duration of action was shorter for acetylcholine than the plant extract.

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1.0 CHAPTER ONE: INITRODUCTION

1.1 Background information

Plectranthus barbatus, a perennial plant that grows in the tropics and subtropics of Africa and India, is one of the most significant species of the genus *Plectranthus* in the family Lamiaceae (Labiatae). Parts of Pakistan, Brazil, China, and Sri Lanka also support its growth. Omoroka (Kisii), *Plecranthus forskohlii* Briq, *Coleus forskohlii* Briq, Mumbu (Digo), and malva santa (Brazil) are some of the other names for the shrub-like plant. The species is also well-known for a variety of traditional purposes, including pesticide use in Kenya and Gabon in addition to herbal medicine. In Yemen, the plant is also used as a vegetable and for conditioning leather and skins, manufacturing compost, animal feed, hastening the maturity of bananas, and more (Mbaria et al, 2023).

Acetylcholinesterase is the enzyme that normally hydrolyzes acetylcholine into choline and acetate in the cholinergic neurons of the CNS and PNS (Aleksandra et al, 2013). The buildup of acetylcholine, hyperstimulation of nicotinic and muscarinic receptors, and disruption of neurotransmission are caused by the enzyme inactivation, which is triggered by a variety of inhibitors. As a result, acetylcholinesterase inhibitors are used as appropriate medications and poisons, with the enzyme functioning as their principal target. Inhibition of acetylcholinesterase therefore increases the accumulation of acetylcholine in the synapse, increasing cholinergic activity. This increases neurotransmission, muscle contraction, cognitive function, and increased gastric motility. An overview of the toxicity and pharmacology of substances that inactivate acetylcholinesterase both temporarily and permanently has also been confirmed (Aleksandra et al, 2013). Special attention has been given to currently licensed medications (donepezil, rivastigmine, and galantamine) used in the pharmacotherapy of Alzheimer's disease as well as

hazardous carbamates employed as insecticides in the case of reversible inhibitors being often utilized in the treatment of neurodegenerative illnesses (Aleksandra et al, 2013)

The mechanism of irreversible acetylcholinesterase inhibition caused by organophosphorus chemicals (insecticides and nerve agents) is next detailed, along with the particular and general harmful effects. Finally, irreversible inhibitors with medicinal applications have been discussed (Colovic, 2013). Additionally, the pharmacological management of organophosphate poisoning with these compounds, with a focus on the administration of oxime reactivators of suppressed enzyme activity as causative medications after the poisoning has been discussed (Mirjana et al, 2014). In addition, before reaching their targets in the nervous system, organophosphorus and carbamate pesticides can be detoxified in mammals through enzymatic hydrolysis. While carbamates are most efficiently broken down by carboxylesterases, organophosphates are most successfully detoxified by being broken down by the matching phosphodiesterase (Lazarevic & Kristic, 2013)

The phytochemistry of Coleus forskohlii and *Plectranthus barbatus* has revealed the presence of tannins, terpenoids, flavonoids, and alkaloids. According to Ganash and Qanash, flavonoids are polyphenolic substances with antioxidizing effects that have been utilized medicinally (Cordeiro et al, 2022). Additionally, recent research indicates that the same feature may be used in clean industrial processes, particularly leather manufacture. Green tea phenolics, ellagic acid, and gallic acid are key components in this chemistry, and their activity in scavenging free radicals has been connected to the antioxidizing properties of plant extracts. A similar antioxidant against free radicals has been discovered for hydroxybenzoic acid. Isoferulic acid, ferulic acid, chlorogenic acid, cinnamic acid, and benzoic acid are further phenolic

compounds of relevance that have only been referenced in traditional medicine (Mbaria et al, 2023).

1.2 Problem statement

Millions of people throughout the world suffer from gastrointestinal illnesses and dysfunctions such as gastroparesis, constipation and other hypomotility disorders, which have a serious impact on their quality of life. The control of digestive functions, including peristalsis and secretion, depends on complex neurotransmitter signaling, with acetylcholine playing a key role. The therapeutic potential of cholinergics in regulating gastrointestinal function has been demonstrated.

Although there are known cholinergics in the market, which are indicated for the management of these conditions, there is a limitation of adverse effects, high cost of these medication, and safety concerns among different patients. Metoclopramide for instance is commonly used prokinetic although with extrapyramidal symptoms. Laxatives like Castor oil has marked diarrhea as a side effect. This study would therefore provide more alternatives to the available therapies, through use of safer and cheaper products obtained from *Plcetranthus barbatus* leaf extract.

1.3 Study justification

Several persons would benefit from the findings of this research directly or indirectly. To begin with, the patients suffering from gastrointestinal diseases, which are uncomfortable, diminish quality of life, and result in high healthcare expenses. Finding treatments that work is essential to enhancing the wellbeing of those who are impacted. Current therapies for gastrointestinal illnesses frequently have drawbacks, such as adverse effects and insufficient

efficacy. To fix these flaws, novel therapeutic strategies are required. The historically used medicinal plant *Plectranthus barbatus* has a reputation for being safe and may contain bioactive substances with the ability to affect gastrointestinal function. Investigating natural remedies is helpful while looking for novel treatments.

Medical practitioners. The prescribers would also have wider options and alternatives to prescribe and dispense to the patients safely and more efficaciously.

Education and research sector. This work promotes interdisciplinary cooperation and knowledge integration, which is crucial for improving healthcare research, by bridging the disciplines of pharmacology and gastroenterology. Understanding *Plectranthus barbatus* anticholinesterase activity in the GIT advances our knowledge of the plant's pharmacological characteristics and its potential as a source for cutting-edge treatments. Traditional medicine has employed *Plectranthus barbatus* for a number of uses. By confirming its promise as a gastrointestinal treatment, indigenous knowledge is preserved while simultaneously modernizing customary methods. Positive results from this study may open the door to more investigation into the development of particular GIT treatments, including the isolation and characterization of active molecules, pharmacokinetic studies, and clinical trials.

Pharmaceutical companies would also benefit from this research as there would be manufacture of the new drugs upon positive discoveries on this study. Additionally, by lowering healthcare expenditures related to digestive problems and opening doors for the pharmaceutical sector, the discovery of new therapeutic molecules from natural sources can be beneficial economically.

1.4 Research questions

Does the methanolic leaf extract of *Plectranthus barbatus* has cholinergic activity in Wister rat's ileum?

How does the cholinergic activity of this extract compare to that of acetylcholine?

Is there a dose response relationship in the cholinergic activity of this methanolic leaf extract?

1.5 Objectives

1.5.1 Broad objective

To investigate the cholinergic activity of methanolic leaf extract of *Plectranthus barbatus* in Wister rats

1.5.2 Specific objectives

To investigate the cholinergic activity of methanolic *Plectranthus barbatus* leaf extract in Wister rats.

To compare the cholinergic activity of *Plectranthus barbatus* methanolic leaf extract with that of acetylcholine

To investigate the dose response in cholinergic activity of *Plectranthus barbatus* methanolic leaf extract

1.6 Study limitations

The results obtained from the ex-vivo animal models may not be extrapolated to the normal humans.

In addition, the exact phytoconstituent in *P. barbatus* that is responsible for the cholinergic activity shall not be established.

Instrument used was not able to fine tune the smooth muscle contractions more vividly.

1.7 Study delimitation

Nearly all the apparatus used in this study were provided by the University laboratory

The plant, *P. barbatus* is common and easily available hence sourcing it from any environment was not a challenge.

1.8 Assumptions

Variations in the plant species in terms of geography, climate, and the plant age are assumed to have a negligible impact in the cholinergic activity of the plant.

1.9 Study significance

This research advances knowledge of *Plectranthus barbatus* pharmacological effects on the GIT. It expands our understanding of natural compounds and their potential therapeutic uses. Positive results can inspire the creation of fresh digestive problems treatment options. Compared to current treatments, these medicines may be more effective and cause fewer side effects. A sizable majority of people suffer from gastrointestinal diseases, and many of them do not respond well to available therapies. Unmet medical needs are addressed in this field of study, which can enhance the quality of life for those who are impacted.

The value of studying natural products is highlighted by the potential of *Plectranthus barbatus*. Further research into plant-based treatments and the preservation of conventional medical knowledge may result from this. The pharmaceutical industry may gain from the discovery of new therapeutic compounds derived from natural sources since it could lower healthcare expenses related to gastrointestinal illnesses. Validating the use of *Plectranthus barbatus* in GIT-related conditions preserves indigenous knowledge, fosters cultural

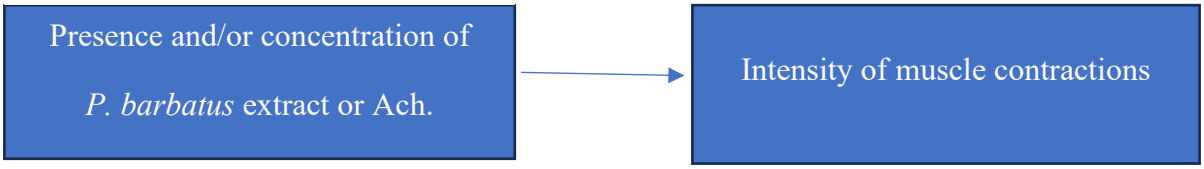
understanding, and modernizes traditional practices. Positive outcomes could prompt more research, such as the identification and characterization of active substances, pharmacokinetic analyses, and clinical trials, with the aim of developing novel GIT treatments.

1.10 Conceptual Framework

Independent variable- Presence and/or concentration of *P. barbatus* extract

Dependent variable- Intensity of muscle contractions

Figure 1 Conceptual framework



1.11 Ethical Considerations

Approval to use laboratory animals was sought from the Department of Pharmacy, Kenyatta University. I applied the three R principles: Replace, reduce, and refine. Rats with physical flaws or those that are ill were swapped out for healthy ones. Rats were only utilized when absolutely necessary. Environmental control and good animal husbandry were used to refine. To lessen suffering and improve animal welfare, appropriate experimental protocols were followed (Smith, 2020). The rats were kept in a holding facility with a relative humidity of 60% and a temperature of 25 degrees Celsius. Animal beddings consisted of untreated wood shavings and vegetables. The animals were handled in accordance with the Institution's Animals Welfare and Ethics Committee guidelines.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

One of the most significant species of the genus *Plectranthus* L' Herit. (Lamiaceae) is *Plectranthus barbatus*, which has a wide range of traditional medicinal uses in Chinese, Brazilian, and tropical African as well as Hindu and Ayurvedic traditional medicine. Therefore, up until now, the plant has been a desirable target for extensive chemical and pharmacological studies. The pharmacology of *Plectranthus barbatus'* components as well as information on its phytochemistry, ethnobotanical applications, and pharmacology are included in this paper (Alasbahi & Matthias, 2013). Several studies have been conducted and articles published on this plant, its phytochemistry, ethnobotanical uses as well as some medicinal properties.

2.2 Phytochemistry Studies

The evaluation of organic compounds in *Plectranthus barbatus* leaf extract revealed significant compounds that were previously used in ethnomedicine, but the results of this study suggested their use in reducing the toxicity of leather and minimizing the contamination of tannery wastewater. In this work, phenolics were extracted, including 4-hydroxybenzoic acid. In related research on phenolic acids, Ganash and Qanash isolated, among other things, caffeic acid, benzoic acid, ferulic acid, gallic acid, and chlorogenic acid using high pressure column chromatography (HPLC) (Ganash et al, 2018).

A recent review by Alasbahi and Melzig documented similar compounds, including but not limited to abietane diterpenoids and 8,13- epoxy-labd-14-en-11-one diterpenoids, for one of the diterpenes discovered in the study, which is illustrated in fig. 3. With the evidence that phenolic acids and other organic compounds were present in the crude leaf extract of

Plectranthus barbatus, it was possible to hypothesize that these compounds may play a role in improving the fixation of chrome when used in combination tanning, as well as increasing the density of bonds surrounding collagen (Alasbahi et al, 2010).

2.3 Anticonvulsant Activity Study

According to earlier research, the hydroalcoholic extract of *Plectranthus barbatus* leaves has anticonvulsant properties that can prevent seizures induced by pilocarpine and strychnine. These drugs are known to be used in induction of seizures in rodents (Rebecca et al, 2014). *P. barbatus* extract did not prevent death, although it did delay pilocarpine-induced seizures in all tested doses. In contrast, animals pretreated with the maximum dose of *P. barbatus* extract (100 mg/kg) before receiving strychnine showed no seizure activity and no signs of mortality. The mechanism underlying strychnine-induced seizures was believed to involve direct antagonistic interactions between strychnine-sensitive glycine receptors in the brainstem, spinal cord, and higher brain regions. This was thought to abolish spinal reflexes and result in motor disturbance, increased muscle tone, hyperactivity of sensory, visual, and acoustic perception, tonic convulsions, and death through respiratory or spinal paralysis or cardiac arrest. These findings showed that therapy with *P. barbatus* partially suppresses strychnine-induced seizures (Luciana et al, 2018).

Pilocarpine injection to mice caused status epilepticus and recurrent limbic seizures, mimicking a number of symptoms of human temporal lobe epilepsy. It has been suggested that *P. amboinicus* extracts may inhibit cholinergic activity at muscarinic receptors. On the other hand*, P. barbatus* extracts have been shown to block acetylcholinesterase activity in the past. *P. barbatus* extracts were administered in the study, although despite delaying the onset of the seizures, they were unable to prevent pilocarpine-induced convulsions. It therefore seems

plausible that the *P. barbatus* extract had a minimal antagonistic effect on muscarinic acetylcholine receptors (Luciana et al, 2018).

2.4 Antimicrobial Activity Study

Another study was conducted to evaluate the potential antimicrobial activity of *Plectranthus barbatus* extract.

By macerating the extracts in 96% methanol, the extracts were created. The crude methanol extract was evaluated using agar perforation methods against the following microorganisms: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella enterica, Streptococcus pneumoniae, Acinetobacter calcoaceticus, Escherichia coli*, and *Candida albicans*. The Minimum Inhibitory Concentration (MIC) was then established using the culture media Brain Heart Infusion (BHI) broth in 96-well plates utilizing the broth micro dilution method. Saline solution 0.9% and Tween 80 were used to solubilize the samples. Ceftriaxone was the active control that was employed. 2,3,5-triphenyl tetrazolium chloride (0.5 mg/ml) was utilized as a developer. Three duplicates of each experiment were carried out. The Kruskal-Walli's test was used for statistical analysis (Regina et al, 2014).

According to the AYRES technique, the extract had an average zone of inhibition of 14,33 (0,47) against Staphylococcus aureus, which is considered active. Staphylococcus aureus (3,12 mg/ml), Staphylococcus epidermidis (6,25 mg/ml), Streptococcus pneumoniae (6,25 mg/ml), and Escherichia coli (6,25 mg/ml) all performed well in the MIC (Ayres et al, 2014). The findings demonstrated that *Plectranthus barbatus* had the capacity to suppress harmful bacteria, indicating that it has antimicrobial activity that offers opportunities for the production

of natural antibiotics. The creation of bioproducts, specifically for infected wounds, is underway, as are their healing capabilities, cytotoxic potential, and bioavailability (Regina et al, 2014)

2.5 Anticholinesterase Activity Study

The purpose of this investigation was to ascertain how *Plectranthus barbatus* (Lamiaceae) herbal tea inhibited the activity of the enzyme acetylcholinesterase (AChE) in the brain. To achieve this goal, laboratory rats were given the herbal tea and its major ingredient, rosmarinic acid, and the impact on the brain AChE activity was assessed. The metabolites of herbal tea were investigated in the plasma and the brain. Both intraperitoneally and intragastrically, the herbal water extract was given. Although brain acetylcholinesterase activity was observed to be inhibited after the plant extract was injected intragastrically, only trace levels of metabolites from *P. barbatus* extract components were discovered in rat plasma (Paulo et al, 2013)

However, when *P. barbatus* extract was given intraperitoneally, rosmarinic acid was discovered in the brain and all of the extract's components were found in plasma. 30 minutes after delivery, the compounds/metabolites were identified in the highest concentrations. 30 and 60 minutes after intraperitoneal treatment, respectively, there was an inhibition of roughly 29.0% and 24.9% in the brain's acetylcholinesterase activity. Given the amount of rosmarinic acid found in the brain, these values were larger than those anticipated, which raised the possibility that other active extract components or metabolites were present in non-detectable concentrations. These findings demonstrate that *P. barbatus* aqueous extract treatment could suppress AChE in the brain (Pedro et al., 2013)

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Site

The experiment was done in Kenyatta University which is at an altitude of about 1795m above sea level, 1.2921^0 S, 36.8219^0 E.

3.2 Study design

Experimental study design was used in this study. In vitro isolated gastrointestinal tissues were used. This was appropriate as the study was carried out in a controlled environment with controlled conditions. It also minimized biasness while conducting the study with strict ethical considerations.

3.3 Materials and equipment

Plectranthus barbatus leaves

Grinder

Crude Methanol

Fractionating column

Volumetric flasks

Round bottomed flasks

Vacuum pump

Distilled water

Buchner funnel

Filter papers, grade one

Labels

Aluminum foil

Analytical balance

DMSO

Transducer

Gas cylinder

Organ bath (Model name/ number: LI-PE-163)

2000ml conical flask

Sodium chloride

Potassium chloride

Calcium chloride

Magnesium chloride

Sodium hydrogen phosphate

Sodium hydrogen carbonate

Glucose

Graph paper

Three rats

Acetylcholine

3.4 Methods

3.4.1 Material Selection and Collection

Under the supervision of a botanist from Pharmacognosy and pharmaceutical Chemistry department, mature healthy leaves of *Plectranthus barbatus* plant were collected from Kenyatta University medicinal garden, Nairobi, Kenya. From an altitude of about 1795m above sea level. 1.2921^0 S, 36.8219^0 E. The leaves were put in polythene sack free from moisture.

Figure 2 P.barbatus plant



3.4.2 Plant Material Processing

The plant leaves were washed to remove the foreign matter, then air dried to constant weight. The dry leaves were then ground into smaller particles just enough for the solvent to penetrate through the cells.

Figure 3 drying process



Figure 4 Grinding



Figure 5 Ground P.barbatus powder



3.4.2 Extract Preparation and Processing

The active phytoconstituents were extracted by maceration process. The extraction solvent was purified by fractional distillation of crude methanol in a fractionating column. The powdered plant material was weighed and placed in a 1000ml conical flask. To this, 1000ml of purified methanol was added and left to stand for 72 hours.

Figure 6 Maceration of the powder

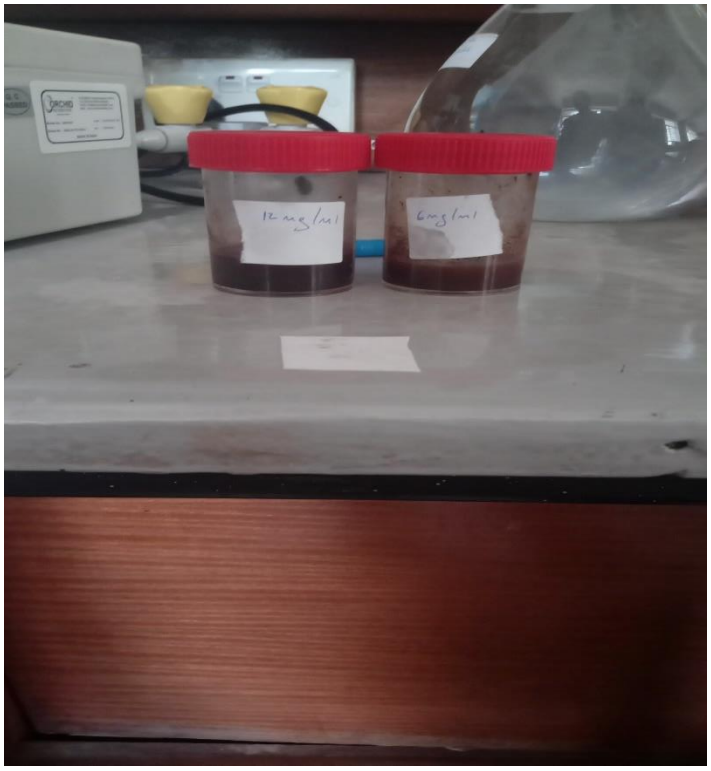


This was decanted and vaporized to remove the solvent. The plant extract remaining in the round bottomed flask was removed and placed in small bottles, left to stand undisturbed for another 24 hours for excess solvent to vaporize leaving a paste in the tube.

3.4.3 Stock Solution Preparation and Dilution

The paste was weighed and dissolved using 5% DMSO and water to make two different concentrations, 12mg/ml and 6mg/ml.

Figure 7 Sample concentrations



3.4.4 Animal Tissue Preparation

Physiological solution was prepared by dissolving various salts in 2000ml of distilled water. Different alts were used in different proportions, 16g sodium chloride, 0.4g potassium

chloride, 0.4g calcium chloride, 0.2g magnesium chloride, 0.1g magnesium hydrogen phosphate, 2g sodium hydrogen carbonate, and 2g glucose. These were measured in 2l volumetric flask and dissolved in enough distilled water then top up to the mark. The transducer and the organ bath were set ready for running the experiment a shown below.

Figure 8 Transducer with the graph cover

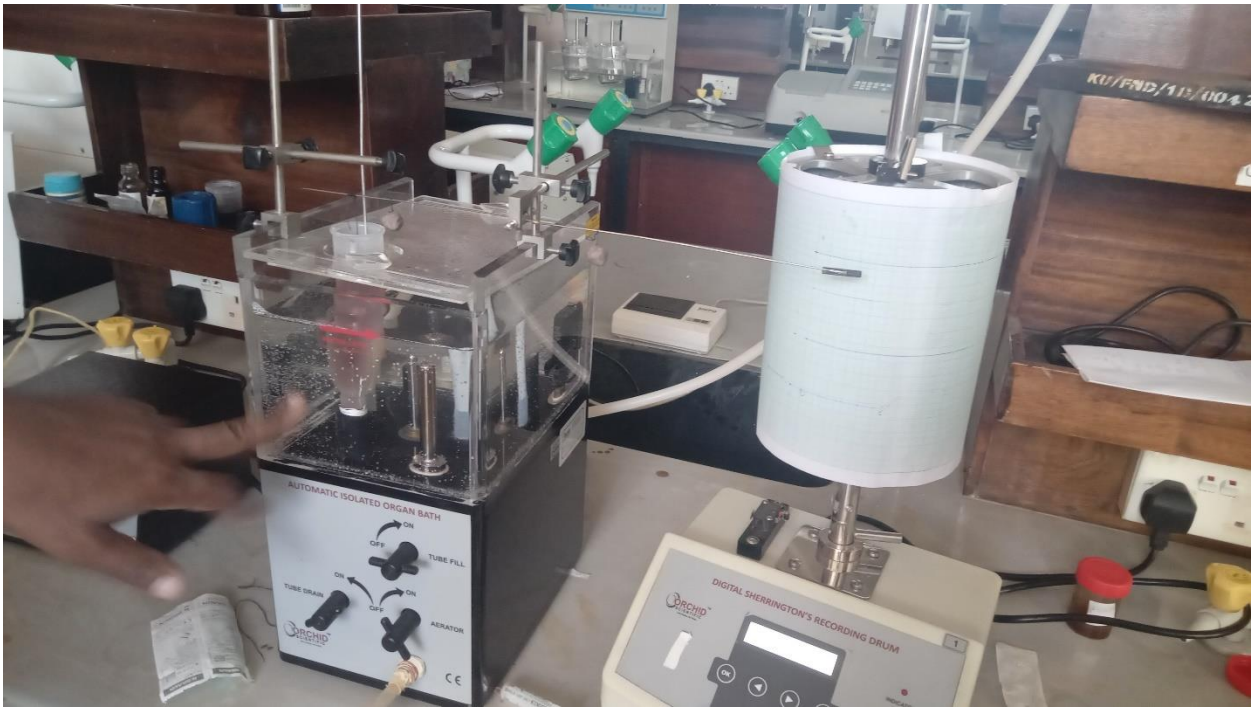


Three Wister rats were sacrificed by euthanasia. Their intestines were extracted and placed in a small volume of the physiological solution to keep the tissues viable and live for a long time.

3.4.5 Tissue Grouping and treatment

The tissues were cut into shorter lengths of about 3cm. the tissue ere the grouped into three different study groups, that is the positive control, and two study groups. Each tissue was tied with a string on both ends. One end was tied fixed in the physiological solution while the other end was tied to the movable pivot of the transducer as in the figure.

Figure 9 Organ bath with sample



3.5 Data Collection and Recording

Strips of each tissue in each group were suspended within the bathing chamber and attached to a force transducer on the other end, one at a time. The transducer was set to run for

60 secs before the sample or control acetylcholine was added to the physiological solution in controlled amounts. The contractions of the smooth muscles of the intestine were recorded on the graph paper connected to the transducer in form of wavs a shown in the previous figures. The system was flushed thrice and refilled with the physiological solution from the input before running the next sample.

3.6 Data analysis

A number of commercially available data collecting software packages were used to capture and analyze data. The parameters of contractions that must be assessed for a precise evaluation of contractile activity are the amplitude of contraction, frequency of contraction, duration of contraction, and mean force integral.

4.0 CHAPTER FOUR: RESULTS AND DATA ANALYSIS

4.1 Results

The total weight of ground leaves of *P. barbatus* was 96.2g

Weight of the paste after solvent extraction was 10.7g

Percentage yield = 10.7/96.2 x 100 = 11.12%

Figure 10 Wave motions before and after addition of 0.01mg/ml acetylcholine.

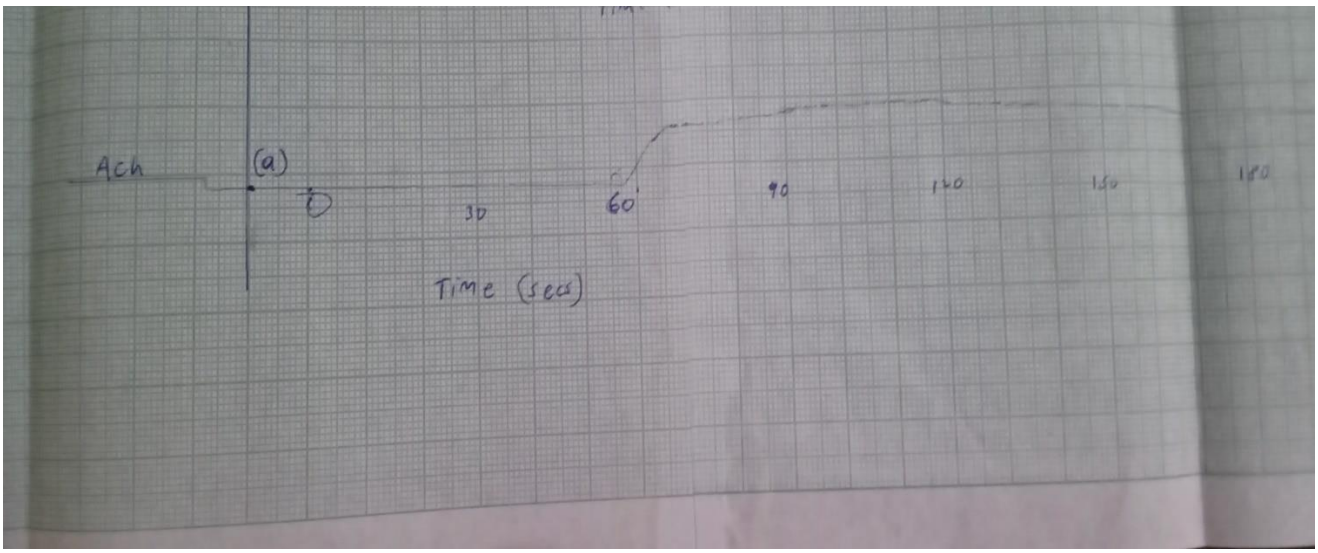


Figure 11 Wave motions before and after addition of 6mg/ml P. barbatus extract

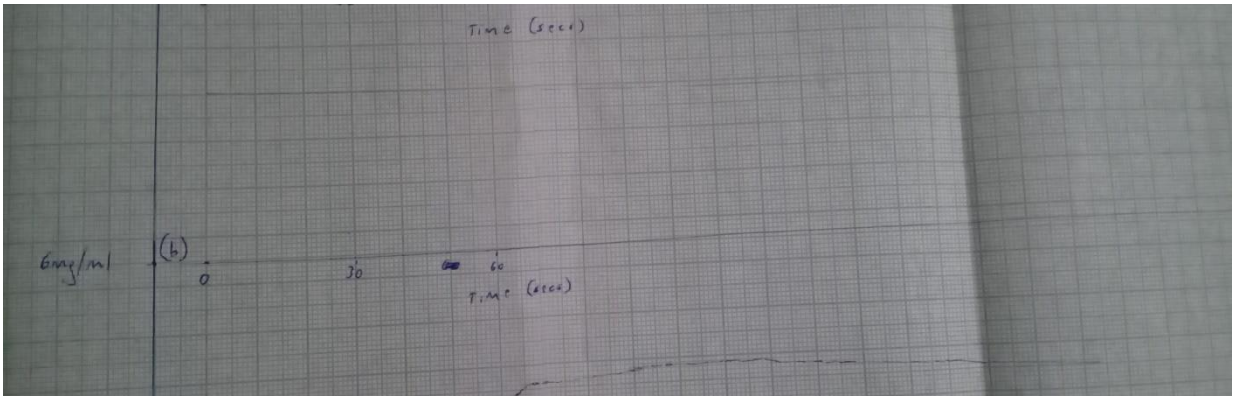


Figure 12 Wave motions before and after addition of 12mg/ml P. barbatus extract

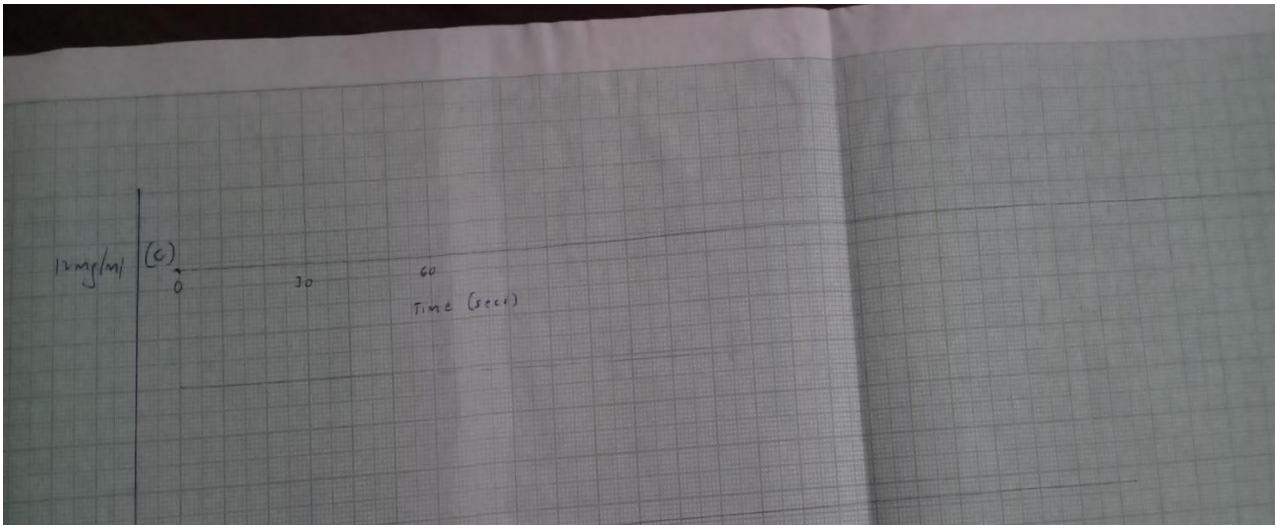
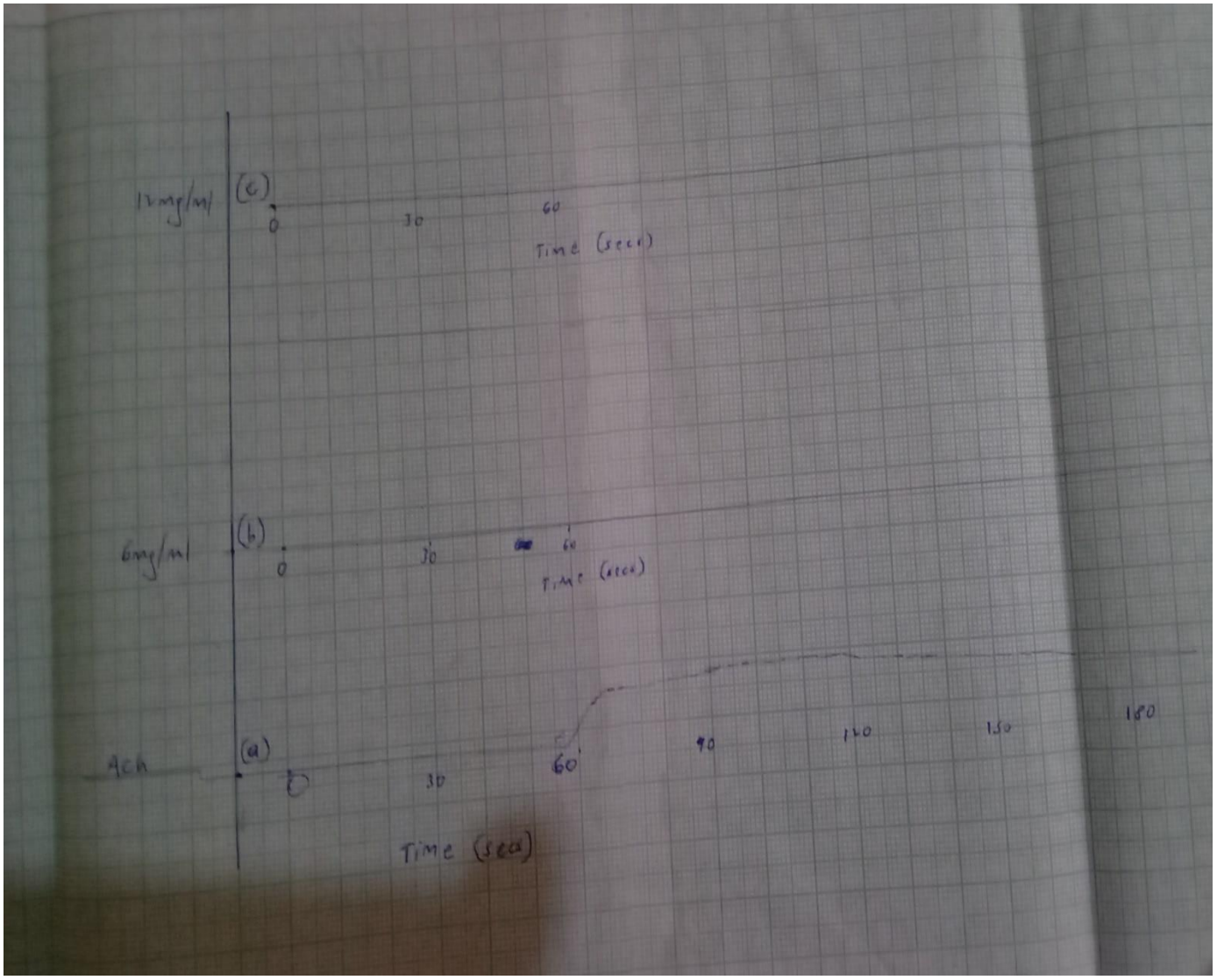


Figure 13 Comparative analysis of the three sample runs



4.2 Data Analysis

The analysis of the above study results was done by plotting simple line graphs for individual samples and a comparison of the two samples with the positive control.

Figure 14 Effect of acetylcholine

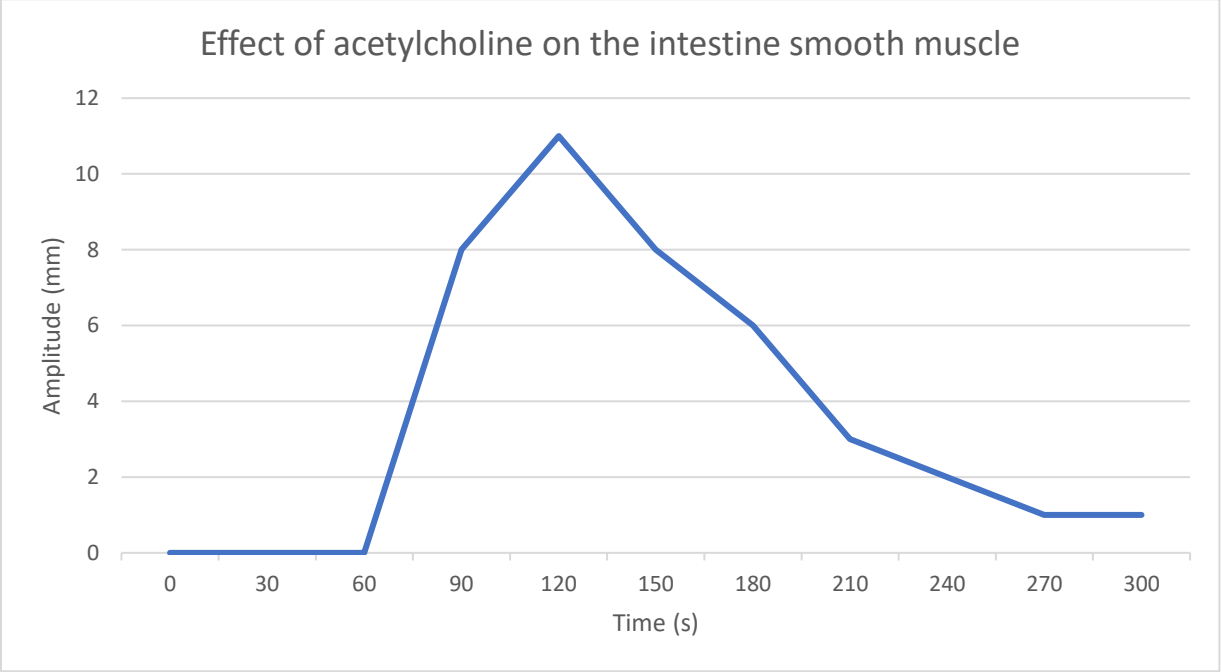


Figure 15 Effect of 6mg/ml sample

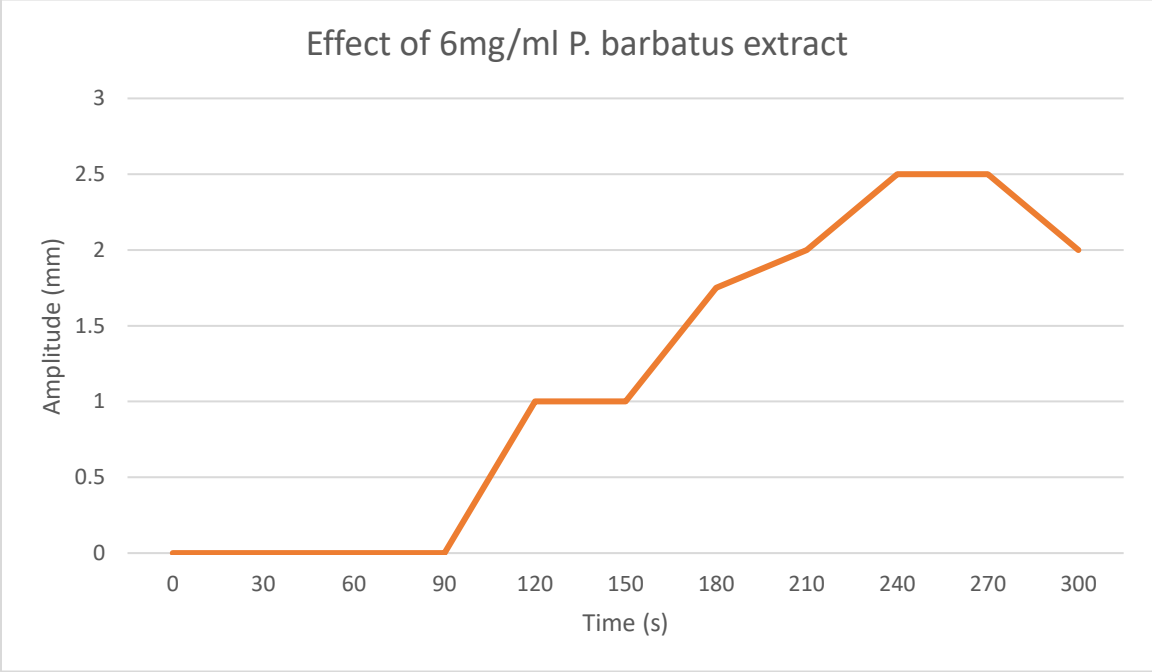


Figure 16 Effect of 12mg/ml sample

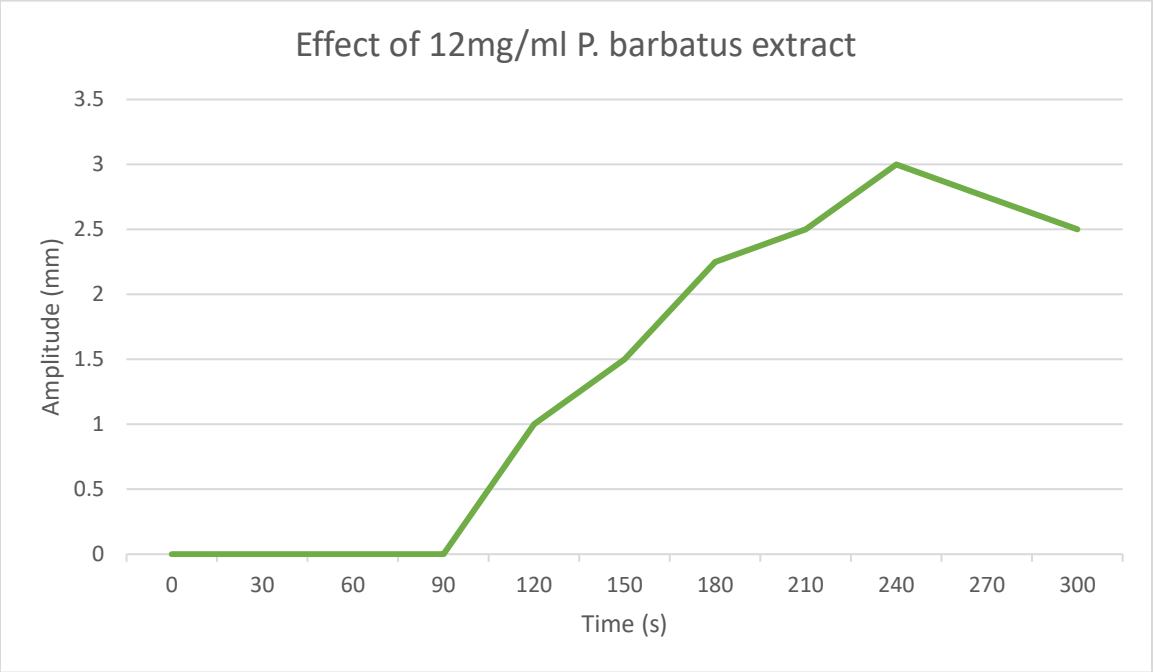
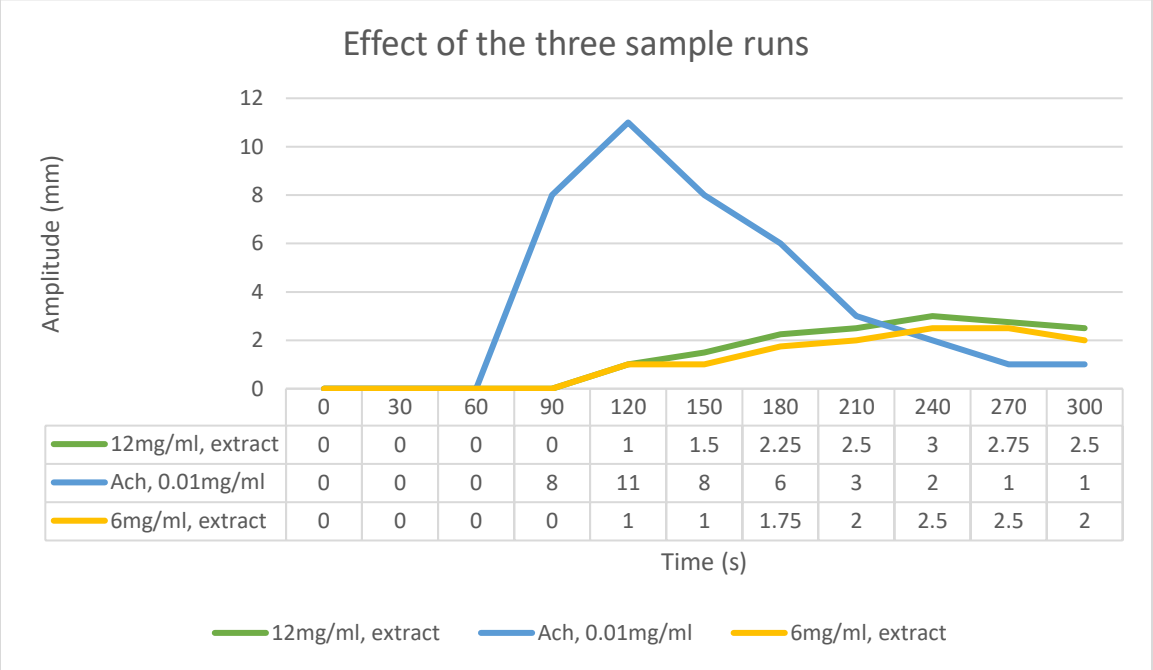
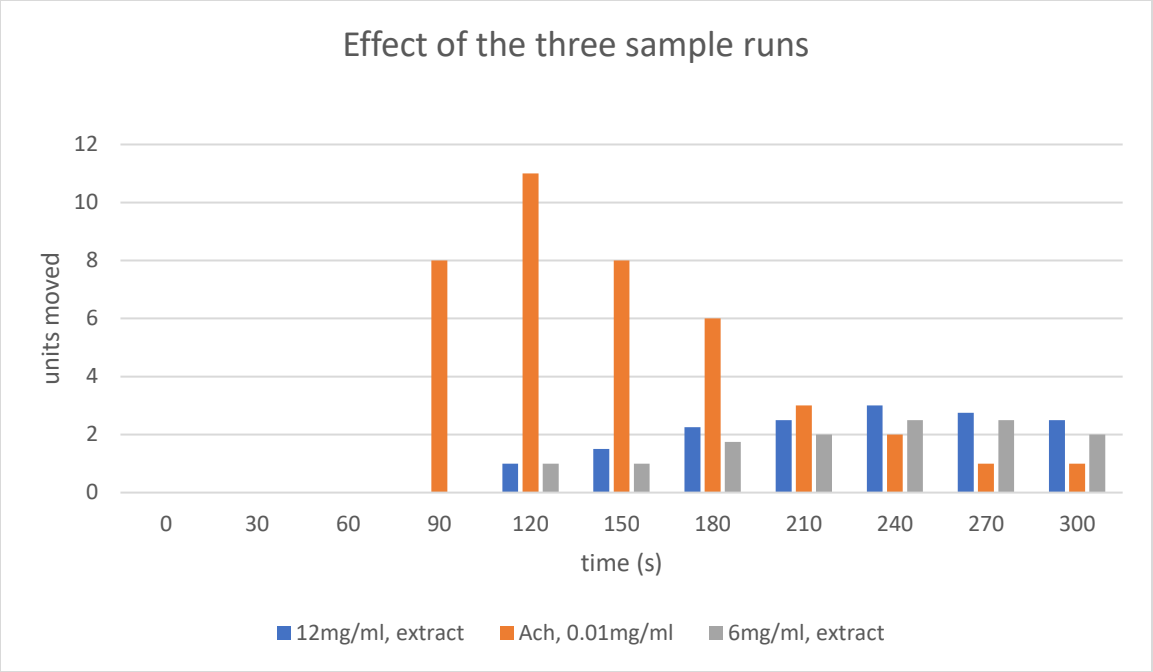


Figure 17 Comparative analysis of samples with control



	0	30	60	90	120	150	180	210	240	270	300
12mg/ml, extract	0	0	0	0	1	1.5	2.25	2.5	3	2.75	2.5
Ach, 0.01mg/ml	0	0	0	8	11	8	6	3	2	1	1
6mg/ml, extract	0	0	0	0	1	1	1.75	2	2.5	2.5	2


Effect of the three sample runs
12
10
8
6
4
2
0
units moved
0
30
60
90
120
150
180
210
240
270
300
time (s)
12mg/ml, extract
Ach, 0.01mg/ml
6mg/ml, extract

5.0 CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The smooth muscle contracts even before the addition of acetylcholine or sample of plant extract. However, this is minimal. The addition of acetylcholine in very small concentrations of 0.01mg/ml produced rapid muscle contraction as shown in figure 14 above. The activation of muscarinic receptors, of which the rat intestinal longitudinal muscle contains M2 and M3 subtypes, mediates the contraction of smooth muscle by acetylcholine. When muscarinic receptors are triggered in single cells, calcium is released from storage, increasing the internal concentration of free calcium and causing calcium-activated potassium channels to open. The voltage-dependent inward calcium current is suppressed by the increase in internal calcium. The opening of channels, which results in membrane depolarization and thereby increases action potential discharge and contraction throughout the muscle, is a third significant consequence (Lim & Bolton, 2017). The onset of action is about 60 seconds and the duration of action is about 210 seconds from this data. Acetylcholine is rapidly hydrolyzed by acetylcholinesterase enzyme hence the short duration of action (Suryanarayanan, 2014). Following the run of acetylcholine with the smooth muscle, the organ bath was flushed thrice to clear the chamber of any small concentrations of acetylcholine before running the next sample of the extract.

P. barbatus leaves extract also produced significant smooth muscle contraction. the first sample was run at a concentration of 6mg/ml. with this, there was a significant contraction of the smooth muscle as illustrated in figure 15. The onset of action was about 90 seconds, with a duration of action of up to 300 seconds. In comparison to acetylcholine, the contraction produced by this sample was much lower than that of acetylcholine but lasted much longer than

acetylcholine. the same kinetics was seen with 12mg/ml sample of the plant extract, though with a little bit more muscle contraction observed with this concentration. See figure 16 above.

Figure 17 provides a comparative analysis of the positive control, and the two samples, all with their effect on the smooth muscles of the intestine. The main mechanism with which *P. barbatus* leaf extract produces smooth muscle contraction is not well known, whether is a direct acetylcholine receptor agonist or an indirect agonist via inhibition of acetylcholinesterase. However, the inhibition of acetylcholinesterase enzyme could be the most probable mechanism based on the longer onset of action and the longer duration of action. Compounds which act indirectly tend to have a longer onset and duration of action (Pedro et al., 2013). There is a clear dose relationship between the two samples of the plant extract. More contractions could be achieved at higher doses.

5.2 Conclusion

This study examined the effects of *Plectranthus barbatus* extract on intestinal smooth muscle, with an emphasis on the effects on contractility. According to our findings, the plant extract caused the smooth muscle to contract significantly, indicating a noteworthy physiological reaction. The contractile impact that has been found is fascinating and calls for more research into the underlying mechanisms. It is crucial to take into account that *Plectranthus barbatus* includes forskolin, a bioactive substance that has been shown to affect cellular signaling pathways, especially those that involve the increase of cyclic AMP levels. The alteration of these intracellular pathways, which results in variations in smooth muscle tone, may be connected to the contraction that was seen.

These results add to the increasing amount of knowledge regarding *Plectranthus barbatus's* physiological impacts and possible uses. Nonetheless, it is imperative to recognize the intricacy of regulating smooth muscle and the want for more investigations to clarify the precise molecular and cellular processes accountable for the detected contraction. Furthermore, a thorough interpretation of the data will require an understanding of the context in which these effects occur, including variations in dosage and experimental circumstances.

5.3 Recommendations

Positive results in this study matter and form a breakthrough for further research to be conducted on the mechanisms by which this plant extract works, the receptor interactions, toxicity studies and pharmacokinetics. All these information may enable the development of a therapeutic intervention for the earlier named diseases from this compound. As a result, the study opens up new directions for future investigations into the effects of *Plectranthus barbatus* on intestinal smooth muscle and gastrointestinal physiology and pharmacology. The properties of this plant extract may be further investigated, which could lead to the creation of innovative therapeutic approaches for diseases involving smooth muscle failure.

REFERENCES

Smith, A. J. (2020). Guidelines for planning and conducting high-quality research and testing on animals. Laboratory Animal Research, 36(1). https://doi.org/10.1186/S42826-020-00054-0

Smith, A. J., Clutton, R. E., Lilley, E., Hansen, K. E. A., & Brattelid, T. (2018). PREPARE: guidelines for planning animal research and testing. Laboratory Animals, 52(2), 135–141. https://doi.org/10.1177/0023677217724823

Ayres MCC, Brandão MS, Vieira-Júnior GM, Menor JCAS, Silva HB, Soares MJS, Chaves MH. Antibacterial activity of useful plants and chemical constituents of the roots of Copernicia prunifera. Vol. 18. Brazilian Journal of Pharmacognosy; 2013

Alasbahi RH, Melzig MF. *Plectranthus barbatus*: a review of phytochemistry, ethnobotanical uses and pharmacology–part 1. Planta Medica. 2010;76(07):653-661.

Cordeiro MF, Nunes TRS, Bezerra FG, Damasco PKM, Silva WAV, M. Ferreira RA, Magalhães OMC, Soares LAL, Cavalcanti IMF, Pitta MGR, et al. Phytochemical characterization and biological activities of *Plectranthus barbatus* Andrews. Brazilian Journal of Biology. 2022; 82:1-12.

Ganash M, Qanash S. Phenolic acids and biological activities of Coleus forskohlii and *Plectranthus barbatus* as traditional medicinal Plants. International Journal of Pharmacology. 2018;14(6): 856-865.

Jing C, Nan Z, Wuyong C, Shiyu, S. Controlling Cr (VI) in Leather: A Review from Passive Prevention and Stabilization of Chromium Complexes. Journal of American Leather Chemists Association. 2017; 112:250-257.

Obiero DK, Ombui JN, Mbaria JM, Yenesew A, Onyuka AS, Kilee TN. Evaluation of chemical compounds in *Plectranthus barbatus* leaves extract for application in reduction of tannery wastewater toxicity. J Phytopharmacol 2023; 12(1):32-38. doi: 10.31254/phyto.2023.12105

Acetylcholinesterase inhibitors: Pharmacology and toxicology. (n.d.). PubMed Central (PMC). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3648782/

Anticonvulsant activity of extracts of *Plectranthus barbatus* leaves in mice. (n.d.). PubMed Central (PMC). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118445/

Antimicrobial activity of *Plectranthus barbatus* (Lamiacea). (n.d.). PubMed Central (PMC). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4210752/

Function of *Plectranthus barbatus* herbal tea as neuronal acetylcholinesterase inhibitor. (2013, January 1). Academia.edu - Share research. https://www.academia.edu/72389664/Function_of_Plectranthus_barbatus_herbal_tea_as_neuronal_acetylcholinesterase_inhibitor

(n.d.). Home. https://phytopharmajournal.com/assets/pdf_files/Vol12_Issue1_05.pdf

Plectranthus barbatus: A review of phytochemistry, ethnobotanical uses and pharmacology - Part 1 - PubMed. (n.d.). PubMed.

SCIRP open access. (n.d.). SCIRP Open Access. https://www.scirp.org/journal/paperinformation.aspx?

https://ilearn.med.monash.edu.au/physiology/gastro-smooth-muscle/simulation#analysis

Sova M. Antioxidant and antimicrobial activities of cinnamic acid derivatives. Mini Reviews in Medical Chemistry. 2012;12:749-767.

Silva FRG, Matias TMS, Souza LIO, Matos-rocha TJ, Fonseca SA, Mousinho KC, Santos AF. Phytochemical screening and in vitro antibacterial, antifungal, antioxidant and antitumor activities of the red propolis Alagoas. Brazilian Journal of Biology. 2019;79(3):452-459.

Mella B, Glanert AC, Gutterres M. Removal of chromium from tanning wastewater and its reuse. Process Safety and Environmental Protection. 2015;95:195-201.

Action of acetylcholine on smooth muscle. (n.d.). PubMed.

Just a moment... (n.d.). Just a moment... https://www.sciencedirect.com/topics/earth-and-planetary-sciences/acetylcholine

APPENDICES

Appendix I: Preparation of physiological solution

PREPARATION OF PHYSIOLOGICAL SALT SOLUTIONS

Quantities for 1 liter

Salts	Locke	Tyrode	Kreb's Hensleit	De Jalon
NaCl	9.0	8.0	6.87	9.0
KCl	0.42	0.2	0.4	0.42
$CaCl_2$	0.24	0.2	0.28	0.06
$MgCl_2$	-	0.1	-	-
$MgSO_4$	-	-	0.14	-
NaH_2PO_4	-	0.05	-	-
$KHPO_4$	-	-	0.16	-
$NaHCO_4$	0.2	1.0	2.1	0.5
Glucose	1.0	1.0	2.0	0.5

For mammalian skeletal muscle use Krebs- Henseleit solution.

For intestines (and most smooth muscles) use Tyrode solution

For heart muscle use Locke solution

For rat uterus use De Jalon solution