Assessment of Uterotonic Potential of *Gossypium Hirsutum* Root Extracts on Isolated Rat Uterus Smooth Muscle

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***Abstract*: Before the development of modern medicine, plants were once the primary source of all medicine in the world, and they continue to provide mankind with new remedies. Uterotonic plants are plants that stimulate uterine contraction and are therefore used to assist labor, remove retained placenta, control postpartum bleeding and as an abortifacient. Gossypium is one of the herbal remedies that is being used to induce labor, despite being used to induce labor the root extracts of *Gossypium hirsutum* had not yet been scientifically studied to assess their uterotonic potential. The aim of this study was to investigate the effect that the root extracts of *Gossypium hirsutum* had on the uterus. This was an experimental study that was conducted using isolated strips of uterine smooth muscles that were obtained from healthy non-pregnant Wistar rats. Extraction of the plant was done by maceration and Soxhlet apparatus using three solvents (aqueous, methanol & petroleum ether). The results of this study showed that crude root extracts of *Gossypium hirsutum* possess demonstrable uterotonic potential, the aqueous extract was found to be the most potent and most efficacious (EC 50 = 7.76 x10-1 mg/ml and E_{max} = 15.25 mN). The extract induced contraction were blocked by three antagonist (Nifedipine, salbutamol and Indomethacin), the antagonism was more with a calcium channel blocker (P= 0.008). The reduced amplitude of contraction in the presence of these antagonist indicated the involvement of beta 2 receptors, prostaglandins and Calcium in the *Gossypium hirsutum* induced uterine contractions.**

***Keywords*. Phytochemical, potency, efficacy, maceration, extraction, uterotonic**

I. INTRODUCTION

Before the development of modern medicine, plants were once the primary source of all medicine in the world, and they continue to provide mankind with new remedies (Van wky et al., 2009). Despite the major breakthrough in science and research, a high percentage of the world's population still rely on herbal medicines. The reliance on herbal medicine has continued mostly in developing countries due to availability, accessibility and affordability of herbal medicine over modern medicine (Omwenga et al., 2012)

According to WHO, herbal medicines are defined as any medicinal product based on herbs, herbal materials, herbal preparations or herbal products that contain as active ingredients parts of plants, plant materials or combinations thereof (WHO, 2013). These herbal medicines are used to treat various ailments, for example women in sub-Saharan Africa, both in urban and rural areas use herbal medicines for the management of gynecological disorders associated with the female reproductive system (Gruber & O'Brien, 2011). Among pregnant women, herbal medicines are used during the prenatal period for the purpose of preparing the uterus and cervix for childbirth, ease pain during labor and enhance endurance during delivery (Tournaire & Theau-Yonneau, 2007).

Uterotonic plants stimulate uterine contraction and are therefore used to assist labor, remove retained placenta, control postpartum bleeding and as an abortifacient (Watcho et al., 2010). Gossypium is one of the herbal remedies that is being used to induce labor (uterotonic plant), it is reported that the roots are used to accelerate labor and childbirth (Rahman, 2014). Despite being used to induce labor, the root extracts of *Gossypium hirsutum* had not yet been scientifically studied to assess their uterotonic potential, this study investigated the effect that the root extracts had on the uterus. The findings helped us understand the continued use of the root extract by folks to induce labor and provided the scientific data on the plant's uterotonic activities. This experimental study was conducted at the University of Zambia, School of Medicine.

II. RESEARCH METHOD

This experimental study was conducted using isolated strips of uterine smooth muscles obtained from non-pregnant healthy female Wistar rats weighing between 160-200g. The extracts used in this study were obtained from the roots of Gossypium hirsutum from Mazabuka, Southern Province of Zambia.

a) Extraction process

The plant roots were washed with tap water to remove dust and dirt, and then rinsed with distilled water. The cleaned plant roots were then cut into small pieces using a clean Axe and dried in the shade for 14 days. After drying, a mortar and pestle were used to reduce the particle size of the sample material, a laboratory blender was then used to reduce the samples further to powder, and the resulting powdered sample material was stored in an airtight container

The dried root (powdered) sample materials were subjected to three types of solvents for extraction; petroleum ether, methanol and distilled water in this order of increasing solvent polarity.

Maceration with distilled water: Extraction using distilled water was carried out by triple maceration for 4hrs with stirring using a magnetic stirrer and heating with a hot plate. The resultant decoction was filtered using whatman's paper and dried in the water bath. The dry extract was stored in the fridge at 4◦C

Extraction using Soxhlet apparatus: The 600ml of petroleum ether was added to the flask and 40g of the sample material was placed within the thimble and then placed within the Soxhlet extractor. The solvent (petroleum ether) was heated using the Isomantle, the heating caused the solvent to starting evaporating and the vapor moved through the apparatus to the condenser. The condensate dripped into the reservoir containing the thimble (with sample extract). Once the solvent level reached the siphon, it poured back into the flask, and the cycle began again. The process ran for a total period of 8 hours after which the resultant extract was dried using a water bath, then stored in the fridge at 4◦C

Extraction using Methanol: 40g of the sample material was extracted using 400ml of methanol by double maceration for 24 hours with stirring using a magnetic stirrer. The extract was then dried using the water bath and stored in the fridge at 4◦C

b) Experimental animals and isolation of uterine muscle

The non-pregnant female Wistar rats weighing between 160 and 200g, housed in standard metal cages,(Figure 1. below) maintained at room temperature (24 ± 2◦C), with 12 hours light/dark cycles, and allowed free access to water and pellet feed were used in this experimental study. The sensitivity of the uterine muscle was induced by pretreating the rats with Stilbestrol (0.2 mg/kg) 24 h before sacrificing them (Ladeji, 2005; Lwiindi, 2015)

Figure 1. The standard metal cages used to keep the rats used in the study.

On the day of the experiment, the rats were humanely sacrificed by cervical dislocation, after which they were dissected and the uteri extracted. The extracted uteri were placed in De Jalon's physiological solution at room temperature and then cut into longitudinal strips that were 2-3 cm long.

Experimental procedure

Longitudinal strips (about 2-3 cm long) of the uteri were mounted in the organ baths containing De Jalon's solution maintained at 37 ± 0.50°C. A thread was attached to one end of the isolated strip of the uterus and tied to the aerator tube in the organ bath containing 25 ml De Jalon's physiological solutions. Another thread was attached to the other end of the isolated uterus and fixed to a lever system fitted on the transducer, which was connected to Power Lab and Lab Tutor (AD Instruments). The tissue tension was adjusted using the transducers to the resting uterine smooth muscle contractions of 5 mN and the force of contraction was zeroed (0 mN) using the Power Lab. The mounted uterine smooth muscle strips connected to the transducers were allowed to equilibrate for 45 minutes in De Jason's physiological salt solution alone, after which contractions were recorded for 10 minutes with distilled water. These initial concentrations were taken as a negative control, thereafter, the effects of graded doses of the root extracts (aqueous, petroleum ether and methanol extract) of *Gossypium hirsutum* were tested in the mounted uterus in the organ bath. The dose-response effects of the *Gossypium hirsutum* root extracts on spontaneous uterus contractions was tested by increasing concentrations of the extract in a non-cumulative manner, allowing 3 washes of the uterus with a fresh physiological salt solution between additions of each concentration, the uterus was allowed appropriate time to recover before subsequent additions of the extracts. The contractions produced by each concentration were observed for 5 minutes and the results were recorded using the Lab Tutor. By comparing the amplitude of the contractions produced by the negative control (distilled water) and the extracts, we were able to determine whether the extract increased, reduced or did not cause any effect in terms of the amplitude of contractions.

The half-maximal effect concentration (EC50) was calculated for each solvent extract and the concentration value of the most potent and efficacious extract was determined and used subsequently. The likely mode of action of the most potent extract was determined by observing the contractions induced by the extract in the presence of antagonists (atropine, nifedipine, prazosin, salbutamol and indomethacin) in the organ bath. If the exact worked through the same receptors as the antagonist, we expected to see a decrease in the amplitude of contractions

Procedure for determination of the likely mode of action of the crude extract of Gossypium hirsutum.

Atropine was added to the organ bath at a dose of 0.225 ml making a final bath concentration of 0.18 mcg/ml, the antagonist was allowed 5 minutes to act with the tissue. Afterwards, the crude extract of *Gossypium hirsutum* was added starting with the smallest dose which had a final organ bath concentration of 0.125 mg/ml, an observation was made

to check if there were contractions occurring, this was followed by three washes with the physiological solution. Atropine was again added at the same dose, while doubling the dose of the crude extract, and the observations were made. This procedure was repeated several times, maintaining the dose of the antagonist while increasing the dose of the extract until the maximum dose of 8 mg/ml as FBC was reached.

The second antagonist to be tested was prazosin which was added to the organ bath making a final bath concentration of 0.4 mcg/ml, it was allowed time to act with the tissue before the addition of the crude extract in a doubling and non-cumulative manner and allowing three washes before the addition of the other doses.

Salbutamol was added to the organ bath in a similar manner with a final bath concentration of 9.6 mcg/ml, this was followed by the addition of the crude extract as described above

Indomethacin was added similarly to other antagonists with a final organ bath concentration of 36 mcg/ml, the addition of crude extract as described above was done.

Nifedipine a calcium channel blocker was also used as an antagonist with a final organ bath concentration of 0.25 mcg/ml and the crude extract was added as described above

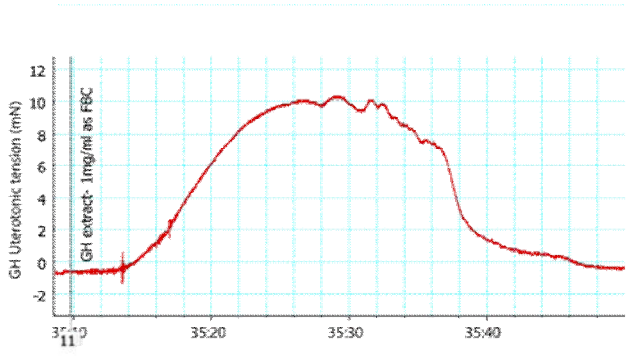
III. RESULTS

The uterotonic potential of crude extract Gossypium hirsutum

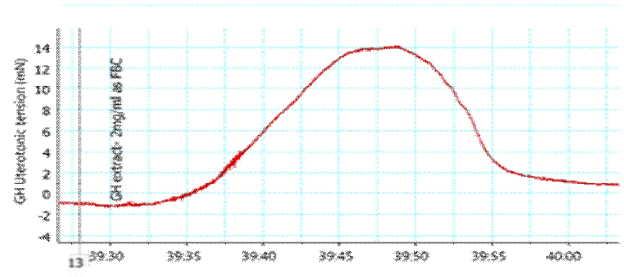
Table 1 below shows the results of uterotonic activity of the three crude extracts of *Gossypium hirsutum*, the responses observed were dose related indicating that the root extract of *Gossypium hirsutum* has uterotonic activitiesTable 1.The effect of *Gossypium hirsutum* root extract on isolated rat uterus smooth muscles

Bath Concentrations of GH dose(mg/ml)	Aqueous extract	Methanol extract	PE extract
	Amplitude (mN)	Amplitude (mN)	Amplitude (mN)
0	0	0	0
0.125	1.46	0.73	0
0.25	5.11	4.42	0
0.5	7.87	4.46	0
1	8.88	6.12	4.21
2	11.14	11.48	6.00
4	13.85	11.29	4.02
8	15.25	11.10	6.05

NB: Non-cumulative concentrations of GH increased the amplitude (strength) of uterine contractions in a dose dependent manner



a)



b)

Figure 2: Contractions induced by the aqueous extract of Gossypium hirsutum. Final bath concentrations of the aqueous extract were a) 1 mg/ml, b) 2mg/ml, and an increase in the dose of the extract resulted in an increase in the amplitude of contraction of the uterus smooth muscles

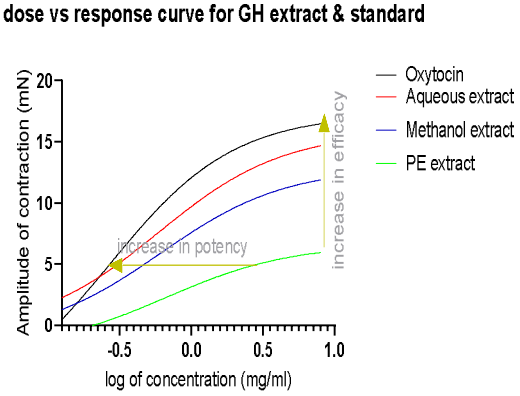


Figure 3: A dose response curve for standard and three solvent extracts of GH on isolated rat uterus showing differences in potency and efficacy. Movement from the right to the left on the dose response cure result in an increase in potency, upward movement result in an increase in efficacy

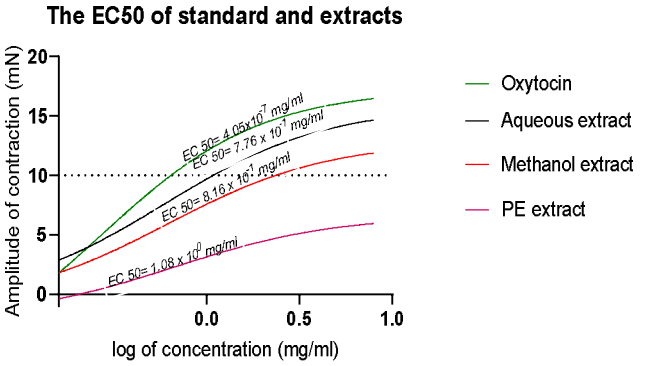


Figure 4: The different EC50 for the standard and solvent extracts of Gossypium hirsutum on isolated rat uterus are shown above

Characterization the pharmacodynamics profile of Gossypium hirsutum on isolated Wistar rat uterine smooth muscles by using standard antagonists

The uterotonic activity of the most potent extract of *Gossypium hirsutum* was evaluated in the presence of antagonists/tocolytic drugs and the results are shown in the figure below.

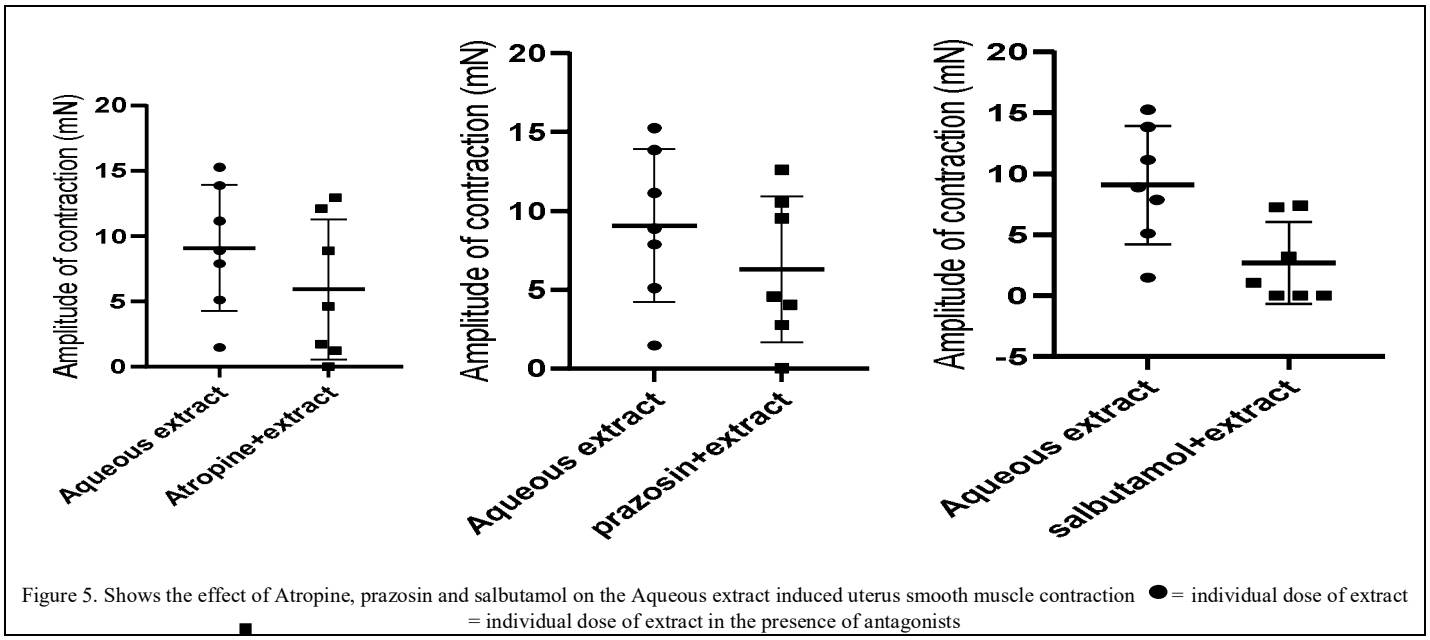


Figure 5. Shows the effect of Atropine, prazosin and salbutamol on the Aqueous extract induced uterus smooth muscle contraction ● = individual dose of extract ■ = individual dose of extract in the presence of antagonists

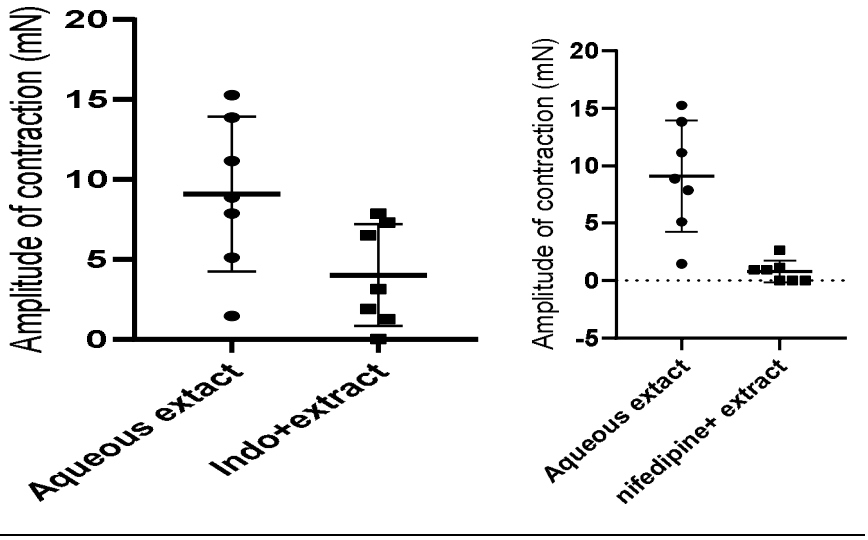
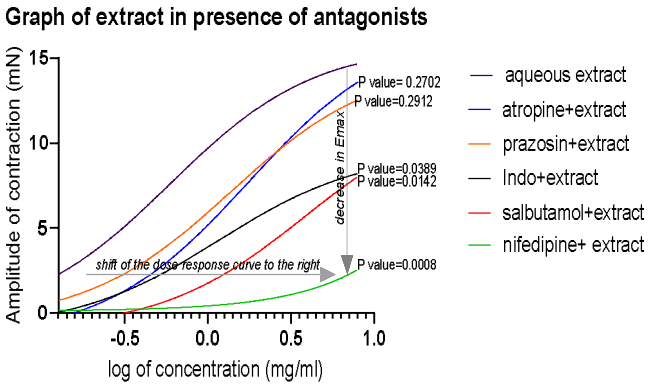


Figure 6. Shows the effect of Indomethacin and Nifedipine on the Aqueous extract induced uterus smooth muscle contraction

● = individual doses of extract

■ = individual dose of extract in the presence of antagonists



A Figure 7.The graph below shows the P values of the dose response curve of the most potent extract in the presence of the antagonists. A shift to the right of the dose-response curve of *Gossypium hirsutum* extract-induced contractions was observed in the presence of Antagonists, a greater shift to the right was observed in the presence of nifedipine as an antagonist.

IV. DISCUSSION

After testing all the three solvent extracts (Aqueous, Methanol and PE extracts) used in this experimental study, their EC50 values were compared, the goal was to determine the most potent solvent extract since potency reflect the sensitivity of the tissue to the drug. The EC50 of Oxytocin which was used as a standard was found to be 4.05x 10^{-7} mg/ml, the EC50 for the aqueous extract was 7.76 x 10^{-1} mg/ml, the EC50 value for the methanol extract was 8.16 x 10^{-1} mg/ml and the EC50 for the PE extract was found to be 1.08 x 10^{0} mg/ml. Oxytocin exhibited higher potency than the aqueous extract this may be attributed to the purity of the drug while the extract was in its crude and un-purified state and contained different components, some of which may even have antagonistic effect (Ayinde et al., 2006). The aqueous extract was the most potent among the crude extracts, it had the EC50 value which was lower concentration than the other two solvent extracts. The methanol extract was the second most potent crude extract and the PE extract was found to be the least potent of the three extracts (Aqueous > methanol > PE). The finding suggests that aqueous extraction removed significant quantities of substances that have uterotonic activities than methanol and PE extraction.

In determining the most efficacious solvent extract, we compared the E_{max} of the three solvent extracts that were used in the study. Efficacy (E_{max}) is said to be the maximum effect which can be expected from a drug (Neubig et al., 2003), the aqueous extract had an E_{max} of 15.25 mN, the methanol extract had an E_{max} of 11.10 mN and the PE extract had an E_{max} of 6.05 mN. The aqueous extract was found to be the most efficacious solvent extract, followed by the methanol extract, and the least efficacious solvent extract was that of the PE. (Aqueous > methanol > PE)

This study has confirmed the uterotonic activity of *Gossypium hirsutum* root extract, the findings are consistent with the reports from the surveys that were conducted on the plant (Rahman & Rogoni, 2014; Tenywa et al., 2020). The extracts obtained using the aqueous solvent were found to be the most potent and most efficacious of the three solvent extracts, the methanol extract was more potent and efficacious than the PE extract which only had activity at higher doses. From these findings, we can conclude that the aqueous solvent was the best solvent that was used to extract active constituents that are more potent and efficacious from the roots of *Gossypium hirsutum*. This study has provided scientific justification for the use of the root by traditional practitioners in maternal healthcare, and specifically for assisting labor. Furthermore, the extract could provide a lead for the development of an active drug that could serve in obstetrics for assisting women with prolonged labor. (Goma et al., 2017).

Determination of the likely mode of action was carried out by testing the uterotonic activity of the crude extract in the presence of the antagonist (atropine, nifedipine, prazosin, salbutamol and indomethacin) in the organ bath. The hypothesis was that if the exact worked through the same receptors as the antagonist, then the amplitude of contractions was going to decrease.

It was observed that with an increase in the dose of the crude extract, the amplitude of contraction also increased, this observation of a dose dependent response occurred even in the presence of atropine and prazosin as antagonist, this observation only occurred at high dose when other antagonists were used.

It was observed that at the lowest dose of the crude extract of 0.125 mg/ml as final bath concentration (FBC) in the presence of atropine, there were no contractions that occurred, the contractions started to occur at a dose of 0.25 mg/ml (FBC) and above this dose an increase in amplitude of contraction was seen with the addition of the doses that followed. Atropine as an antagonist gave a 15.15 % decrease on the E_{max}, and when the dose response curve graph was plotted a slight shift of the curve to the right was observed. There was a slight change in the EC50 with a P value was 0.2702 ($P > 0.05$) which meant that the change in the amplitude of contraction in the presence of atropine was not significant. The extract may not have been achieving its action through the use of muscarinic receptors, similar findings were also reported on a study that was assessing the uterotonic activity of *Helichrysum mechowianum* (Helymext) where they found that no inhibition occurred in the presence of atropine (Eyi Minsta et al., 2017)

When prazosin was used as an antagonist the activity again started at a dose of 0.25 mg/ml, similar to the dose at which the activity started when atropine was used as an antagonist. The E_{max} decreased by 17.31% and a slight shift to the right of the dose response curve was observed. There was also a slight change in the EC50 and the P value was 0.2912 ($P > 0.05$), this indicated that the decrease in the amplitude of contraction was not significant. So, in the presence of prazosin as an antagonist, the crude extract was still able to induce significant contractions, the extract was able to induce

contractions using other receptors that are not blocked by prazosin. The alpha 1 receptors seemed not to be involved in the *Gossypium hirsutum* induced contractions.

Using salbutamol as an antagonist, a significant change to the amplitude of contraction was observed. There was no activity observed at lower dose of the crude extract, the activity only started at a high dose of 1 mg/ml as final bath concentration. A significant shift to the right of the dose response curve was observed, the E_{max} decreased by 51.61%, the EC 50 increased to 2.55 mg/ml and the P value was 0.0142 ($P< 0.05$), these changes occurred when an agent that relaxes the beta 2 receptors (salbutamol) was present in the organ bath. The contraction induced by the extract were significantly reduced, the EC50 tripled and the E_{max} reduced to half. These findings led to the assumption that the crude extract of *Gossypium hirsutum* may have achieved its action through the involvement or use of the beta 2 receptors.

With indomethacin as an antagonist it was observed that there was a significant shift to the right of the dose response curve, a shift similar to the one that occurred when salbutamol was used as an antagonist. The E_{max} reduced by 48.33% and the EC50 increased to 1.08 mg/ml, with a P value of 0.039 ($P< 0.05$). These findings were an indication that the prostaglandins were involved in the contractions that were induced by the crude extract of *Gossypium hirsutum*.

No activity was observed at smaller doses of the crude extract in the presence nifedipine, the activity only started at a dose of 1 mg/ml as final bath concentration. A very significant shift of the dose responses curve to the right was observed, the E_{max} decreased by 82.62%, and the EC50 was increased to 65.92 mg/ml with a P value of 0.0008 ($P < 0.01$). The observation of a very big increase to the EC50 in the presence of a calcium channel blocker and a very significant drop of the E_{max} is suggestive of a possible involvement of calcium mobilization in the activity of the extract. These findings are similar to observations made while studying the contraction induced pomegranate (*Punica granatum L., Punicaceae*), the seed extracts showed an extracellular calcium dependency (Promprom et al., 2010).

In summary, when salbutamol was used as an antagonist, most of the doses were only able to produce an amplitude less than 5 mN, it was only the higher doses that went above the amplitude of 5 mN (figure 5). A similar observation was made when Indomethacin was used as an antagonist, more than half of the given doses gave an amplitude of less than 5 mN. Nifedipine on the other hand showed a very strong inhibition, most of the doses gave an amplitude of less than 2 mN, it was only the highest dose that was able to exceed the amplitude of 2 mN, but it still could not go above 5 mN (figure 6), this signifies that the calcium channel blocker had the strongest inhibitory activity

The reduced amplitude of contraction in the presence of the antagonist (salbutamol, indomethacin & nifedipine) indicated the involvement of beta 2 receptors, prostaglandins and Ca^{2+} in the *Gossypium hirsutum* induced uterine contractions, this meant that the crude extract either contained multiple compound or worked through different receptor sites. Further studies should be conducted on the extract of *Gossypium hirsutum*, the studies should include purification, chemical characterization and optimization of the active constituents.

V. CONCLUSIONS

Root extracts of *Gossypium hirsutum* possess demonstrable uterotonic potential, with the aqueous extract being the most potent and most efficacious. The extract induced contraction were blocked by three antagonist (Nifedipine, salbutamol and Indomethacin), the antagonism was greater with a calcium channel blocker ($P= 0.008$). The reduced amplitude of contraction in the presence of these antagonist indicated the involvement of beta 2 receptors, prostaglandins and Ca^{2+} in the *Gossypium hirsutum* induced uterine contractions.

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