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Phytochemical Profiling, Toxicity Study and Abortifacient Activity of Seed and Whole Plant of *Momordica charantia* Linn. (Cucurbitaceae)

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ABSTRACT

The objective of this study was to extract the seed and plant of *Momordica charantia* Linn. with methanol using soxhlet apparatus, phytochemically screen the methanol extracts using standard procedures, determine the acute toxicity of the methanol extracts of seeds and plant in Wistar strain rats by Lorke's method and evaluate the abortifacient activity on adult nonpregnant albino rat *in-vitro*. The phytochemical screening of both the methanol seed and plant extracts revealed the presence of carbohydrates, terpenoids, cardiac glycosides, cardenolides and saponins for seed, while carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins and flavonoids were present in the plant extract. The intraperitoneal LD_{50} of seed and plant extracts were calculated to be 288 mg/kg and 714 mg/kg respectively. The abortifacient activity of *Momordica charantia* of both plant and seeds were investigated in induced contraction on the uterus in an organ bath setup. Oxytocin was used as a standard. The seed and plant extracts in a dose dependent manner induced contraction, the amplitude of contraction and percentage increase were independently significant ($p<0.05$). The result showed the synergistic activity between oxytocin and the plant extract. It may be concluded that the methanol seed and plant extracts of *M. charantia* induced uterine contraction, thus having abortifacient activity and this justifies this claim in traditional medicine.

1. Introduction

Pregnancy is defined as the period between conception (260–294 days since the first day of the last menstrual period) to birth time. Newborn before the interval of complete 37 weeks are called preterm and those born after 42 weeks or beyond are called post-term. Abortion is an act of deliberate termination or cessation of pregnancy. Miscarriage is also termed a spontaneous abortion which is the spontaneous loss of pregnancy before the 20th week which is usually painful both emotional and physical.

Miscarriage is one of the most frequent problems that happens during pregnancy in the human [1]. Some chemical substances, plants, beverages, drinks and other consumables to extent have the potentials of causing miscarriage during pregnancy. These substances which include coffee (containing caffeine) consumption during pregnancy have been associated, in some studies, with miscarriage. Scientifically, there is no safe dose regimen or limit for alcohol during pregnancy, though the mechanism of this substance in miscarriage is still less-known. Momordica charantia or bitter gourd is known to be domesticated in India and China. Momordica charantia grows in most tropical parts of the world, which include Eastern and Western Africa, Asia, the Caribbean and South Africa. It grows well during the rainy season in Nigeria. It is found mainly in home gardens and farmlands as a troublesome weed [3]. The plant has been used for long

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in the past by the people of Amazon, the leaf tea is used for the management of diabetes, soreness, wounds and infection. It is also used to treat measles, hepatitis and fever. In Africa and Asia, the plant is used traditionally to enhance digestion, metabolism, blood circulation and immunity. In Philippines, the plant is used to lower high blood sugar levels. The popularity of *Momordica chantia* in various traditional system of medicine for several ailments as an abortifacient, anthelmintic, antidiabetic, contraceptive, antidysmenorrhoeal, treatment of eczema, as an emmenagogue, fever, managing leucorrhoea, piles, pneumonia, psoriasis, as a purgative, in treating rheumatism and scabies [4].

The prevalence reports of pregnancy abortion in Nigeria has resulted in the loss of many lives, both the young and older women of childbearing age. The plant *Momordica charantia* Linn. has been used locally in a soup which been consumed by pregnant women in North-eastern Nigeria. This has become paramount to investigate the toxicity and abortifacient effect of this plant in order to establish scientifically, its dosage regime as well enlighten the general public of the effect of the plant.

2. Materials and Methods

2.1 Sample Collection, Identification and Preparation

The parts of the plant used in this research project were the whole plant and seed of *Momordica charantia* Linn. which were collected within the University of Maiduguri Campus. It was identified by a Plant Taxonomist at the University of Maiduguri, Maiduguri, Borno State. A voucher specimen number: UMC/FP/DP/342 was given and the specimen of the sample was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Borno State. The seed and whole plant were air-dried at room temperature under shade, gound into powdered form using wooden mortar and pestle. The powdered seed and whole plant were stored separately in an air-tight container prior to extraction.

2.2 Extraction of Plant Materials

Each plant materials (250 g) were introduced into a conical flask of the extractor in one liter distilled water. An analar grade methanol (90%) was transferred into the flask containing the powdered seeds and whole plant and shaken. The mixture obtained was refluxed for two (2) hr, decanted, filtered and dried in an oven at 40^0 C.

2.3 Phytochemical Screening

The seed and plant extracts were subjected to qualitative phytochemical screening to test for the presence of alkaloids, flavonoids, saponins, glycosides, terpenes/terpenoids, fatty acids, resins, etc. as described by Evans [5].

2.4 Experimental Animals and Acclimatization

Thirty adult albino rats of both sexes weighing 80-280 g were used for both the acute toxicity studies (LD_{50} evaluation) and the abortifacient activity. These animals obtained from the Animal House section of the Faculty of Veterinary Medicine, University of Maiduguri, Borno state. The animals hosed in plastic cages in the animal section of the Physiology, Pharmacology and Biochemistry Laboratory of the Faculty of Veterinary Medicine.

The animals were kept in plastic cages at the standard condition of temperature and light and humidity for period of two weeks to allow them to acclimatize to laboratory conditions. The animals were allowed water *ad libitum* and standard livestock feed (Grand Cereals and Oil Mills Ltd.) Bukuru, Jos, Plateau State Nigeria and were handled according to the International Guiding Principles for Biochemical Research Involving Animals [6].

2.5 Acute Toxicity Studies (LD_{50} Determination)

The acute toxicity (LD_{50}) of the methanol seed and plant extracts of *M.charantia* were determined individually using the method described by Lorke [7]. The experiment was divided into two phases by the use of intraperitoneal route of administration and is described as follows;

In Phase I, nine (9) healthy Wistar strain albino rats of both sexes weighing 95-180 g were randomly selected and divided into three groups (labeled A, B and C) of three animals each. The animals in each group were weighed and labeled with marker on either head, back, or tail (as required), as a mark of identification. The groups were treated respectively with the extract at an incremental dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg intraperitoneally. The animals were then observed for 24 hours for signs of toxicity and mortality. In Phase II, four (4) healthy Wistar strain albino rats of both sexes weighing 95-180 g were randomly selected and divided into four groups A, B, C and D one animal each, weighed and marked. The animals were then given graded doses of seed extract intrapeitoneally 140 mg/kg, 225 mg/kg, 370 mg/kg and 600 mg/kg respectively and graded doses of plant extract intraperitoneally 140 mg/kg, 225 mg/kg, 370 mg/kg, 600 mg/kg and 850 mg/kg respectively based on the result of phase I each. The rats were allowed access to food and water *ad libitum* and were observed for 24 hours for signs of toxicity and death after which the LD_{50} was calculated.

2.6 Abortifacient Activity of Methanol Seed and Plant Extracts of Momordica charantia Linn.

Preparation of Uterine Tissues

Twenty (20) mature non pregnant female albino (10 for each extract) weighing 120-280 g rats were humanely sacrificed by cervical dislocation and the uterine horns were rapidly excised and carefully cleaned of the surrounding connective tissues and opened along the mesenteric border. The uteri were isolated and immediately placed in the physiological saline solution. To measure the force, a uterine strip was attached at the end of each metal hooks, with one hook being attached to one chamber physiograph to each transducer (Meditech Technologies India Private Limited) under a resting tension of 1 g in an organ bath containing Tyrode physiological salt solutions of the following: NaCl, KCl, glucose, $NaHCO_3$ and $CaCl_2$ gassed with 100% oxygen and maintained at 37^0 C, pH 7.4. The tissue 1 g tension was allowed to equilibrate for one hour before addition of the extract or the oxytocin. The electrical signal from the transducer was amplified and converted to a digital signal and recorded on the computer using Chart software [8].

2.7 Chemicals and Physiological Solutions

The solvents and chemicals used were of analytical grade and obtained from Sigma Chemical Limited**.** All stock solutions were prepared and stored in accordance with the guidelines of the manufacturer. The physiological salt solution with pH 7.4 was prepared with the following composition (g/L): NaCl (9.0), KCl (0.42), glucose (0.50), $NaHCO_3$ (0.50) and $CaCl_2$ (0.06). Oxytocin was used at a concentration of 1ug/mL to produce a phasic concentration.

2.8 Induction of Contraction of the Rat Uterine Strips on Organ Bath Set-up and Record on Physiograph

Albino rat uterine strip of approximately 2 cm was threaded and mounted on a clean organ bath containing 75 ml of Tyrode physiological solution. The transducer was previously calibrated and non-drug induced amplitude of contraction (normal contraction) was recorded as N. The uterine was allowed to stabilize for a few minutes before the methanol plant extract of *Momordica charantia* was added to the bath at concentrations of 0.2, 0.4 and 0.6 mg/ml respectively and the amplitude of contraction was recorded on the physiograph respectively.

Albino rat uterine strip of approximately 2 cm was threaded and mounted on clean organ bath containing 75 ml of Tyrode physiological solution. The transducer was previously calibrated and non-drug induced amplitude of contraction (normal contraction) was recorded as N. The uterine was allowed to stabilize for few minutes before the methanol seed extract of *Momordica charantia* was added to the bath at concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml respectively and the amplitude of contraction was recorded on the physiograph respectively.

Albino rat uterine strip of approximately 2 cm was threaded and mounted on clean organ bath containing 75 ml of Tyrode physiological solution. The transducer was previously calibrated and non-drug induced amplitude of contraction (normal contraction) was recorded as N. The uterine was allowed to stabilize for few minutes before the oxytocin was added to the bath at concentrations of 0.2, 0.4 and 0.6 ug/ml respectively and the amplitude of contraction was recorded on the physiograph respectively.

Albino rat uterine strip of approximately 2 cm was threaded and mounted on clean organ bath containing 75 ml of Tyrode physiological solution. The transducer was previously calibrated and non-drug induced amplitude of contraction (normal contraction) was recorded as N. The uterine was allowed to stabilize for few minutes before the methanol plant extract of *Momordica charantia* and oxytocin were added to the bath at concentrations of 0.2, 0.4 and 0.6 mg/ml for the extract and 0.2, 0.4 and 0.6 ug/ml for oxytocin respectively and the amplitude of contraction were recorded on the physiograph respectively.

2.9 Statistical Analysis

Data obtained from this study were expressed as Mean ± Standard Deviation (S.D) and analysed by one-way analysis of variance (ANOVA) to determine the relationship between the variable means using Statistical Package for Social Sciences (SPSS) version 16.0 and P-value<0.05 was considered significant.

3. Results and Discussion

3.1 Percentage Yield and Physical Appearance of the Extract

The weight, colour, texture and the percentage yield of the aqueous seed and plant extracts of *Momordica charantia* from reflux extraction are presented in Table 1. The weight of the seed extract and the whole plant were 32.16 g and 60.30 g respectively, the colour of the extracts were dark brown and dark green for the seed and plant extracts respectively, the texture for both were gummy while the percentage yield were 26.8 % $^w/_w$ and 24.12 % $^w/_w$ respectively.

3.2 Phytochemical Screening of Methanol seed and plant extracts of Momordica charantia

The phytochemical screening of both the methanol seed and plant extracts revealed the presence of carbohydrates, terpenoids, cardiac glycosides, cardenolides and saponins for seed, while carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins and flavonoids were present in

the plant extract. The result of the studies was shown as presented in Table 4.2.

Table 1: Extraction Profile of Methanol Seed and Plant Extract of *Momordica charantia* Linn.

Parameter	Methanol Seed Extract	Methanol Plant Extract
Weight	32.16 g	60.30 g
Colour	Brown	Dark green
Texture	Gummy	Gummy
Percentage yield	26.80	24.12

Table 2: Phytochemical Screening of Methanol Seed and Plant Extract of *Momordica charantia*

Test	SE	PE
Test for carbohydrate		
Molish's test	+	+
Test for monosaccharides (Barfoed)	-	-
Test for free reducing sugar (Fehling)	+	+
Test for Soluble starch	-	-
Test for cardiac glycosides		
Salkwoski's test	+	-
Lieberman-Burchard test	+	+
Test for Glycosides		
Test for anthraquinones	-	-
Test for flavonoids	+	-
Shinoda's test	+	-
Sodium hydroxide test	+	-
Tannins		
Ferric chloride test	-	-
Lead acetate test	-	-
Test for Phlobatannins	-	-
Test for cardenolides		
Keller-Killiani's test	+	+
Test for Saponins		
Frothing test	+	+
Test for Terpenoids	+	+

Key: (+) = Present; (-) = Absent; SE = Seed Extract; PE = Plant Extract

3.4 Acute Toxicity Studies of Methanol Seed and Plant Extracts of Momordica charantia

The result for LD_{50} is shown in Tables 3 and 4. The intraperitoneal LD_{50} of seed and plant extracts were calculated to be 288 mg/kg and 714 mg/kg respectively.

3.5 Concentration-response Effect of the Methanol Seed and Plant extract of Momordica charantia on Amplitude of Contraction of Rat Uterine Strips

Methanol plant extract of *Momordica charantia* induced spontaneous contractions of albino rat uterus upon exposed to a graded bath in a concentration-dependent manner. Methanol plant extract of *Momordica charanita* showed the highest amplitude of contraction (6.26 ± 0.77) when compared to methanol seed extract of *Momordica charantia* with amplitude contraction of 5.48 ± 1.3 at 1.0 mg/ml as shown in Fig. 1 and 2 and presented in Table 5 and 6.

3.6 Concentration-response Effect of the Oxytocin on Amplitude and Frequency of Contraction of Rat Uterine Strips

Oxytocin was found to cause an increase in the amplitude of contractions of albino rat uterus exposed to a graded bath in a concentration-dependent order from 6.00 ± 0.53 to 6.30 ± 0.53 at 0.2 and 0.6 mg/ml with a % increase in uterus contraction of 28.0 and 34.0 respectively as shown in Fig 3. The results were calculated and presented in Table 7.

3.7 Concentration-response Effect of the Methanol Seed and Plant extract of Momordica charantia + Oxytocin on Amplitude of Contraction of Rat Uterine Strips

A recipe of seed and plant extract of *Momordica charantia* and oxytocin revealed an increased in both amplitude and frequency of contraction of albino rat uterus exposed to a graded bath in dose-dependent manner as shown in Fig. 5. The results were calculated and presented in Table 8.

Table 3: Intraperitoneal acute toxicity (LD_{50}) test of the methanol seed extract of *Momordica charantia* Linn.

Phase	No. of Rats	Dose (mg/kg)	Clinical Sign	Mortality
1	3	10	None	0/3
1	3	100	None	0/3
1	3	1000	None	3/3
2	1	140	None	0/1
2	1	225	None	0/1
2	1	370	None	1/1
2	1	600	None	1/1

i.p $LD_{50} = \sqrt{a \times b}$; =288 mg/kg

Where a= least dose that kills the animal = 1/1; b= highest dose that does not kill the animal= 0/1.

Table 4: Intraperitoneal acute toxicity (LD_{50}) test of the methanol plant extract of *Momordica charantia* Linn.

Phase	No. of Rats	Dose (mg/kg)	Clinical Sign	Mortality
1	3	10	None	0/3
1	3	100	None	0/3
1	3	1000	None	3/3
2	1	140	None	0/1
2	1	225	None	0/1
2	1	370	None	0/1
2	1	600	None	0/1
2	1	850	None	1/1

i.p $LD_{50} = \sqrt{a \times b}$; =715 mg/kg

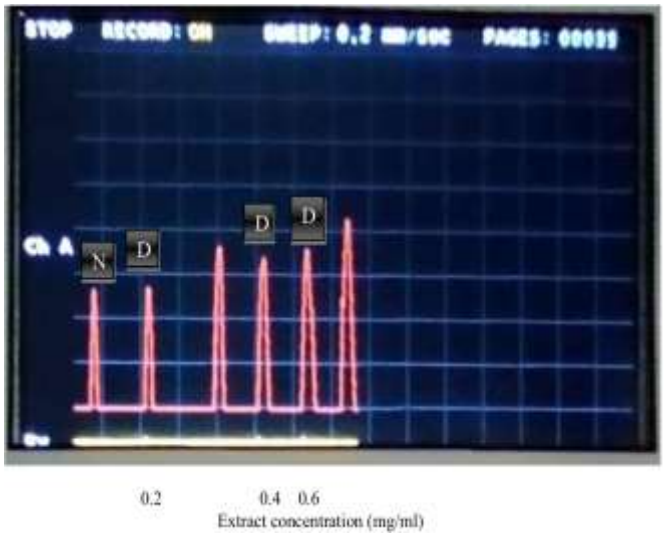

Figure 1: Methanol plant extract of *Momordica charantia* induced contraction amplitude in dose-dependent manner on albino rat uterus *in-vitro* (100 mg/ml)
Key: N= Normal contraction (without drug); D= Drug induced contraction

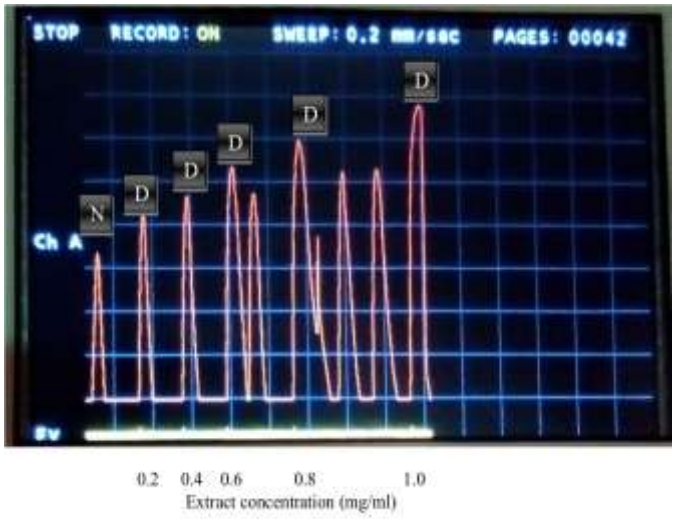

Figure 2: Methanol seed extract of *Momordica charantia* induced contraction amplitude in dose-dependent manner on albino rat uterus *in-vitro* (100 mg/ml)
Key: N= Normal contraction (without drug); D= Drug induced contraction

Table 5: Amplitude of contraction of albino rat uterus exposed to graded bath concentrations of methanol plant extract of *Momordica charantia* (100 mg/ml)

Extract concentration (mg/ml)	Bath concentration (mg/ml)	Normal segment (cm)	Extract treated segment (cm) ± S.D	Difference in amplitude	% increase
0.20	0.27	2.80	2.80 ± 0.40	0.00	0.00
0.40	0.53	2.80	3.50 ± 0.40	0.70	25.00
0.60	0.80	2.80	3.60 ± 0.40	0.80	28.60

Standard deviation (S.D) of plant extract treated segment = ± 0.40

Table 6: Amplitude of contraction of albino rat uterus exposed to graded bath concentrations of methanol seed extract of *Momordica charantia* (100 mg/ml)

Extract concentration (mg/ml)	Bath concentration (mg/ml)	Normal segment (cm)	Extract treated segment (cm) ± S.D	Difference in amplitude	% increase
0.20	0.27	3.30	4.20 ± 1.30	0.90	27.30
0.40	0.53	3.30	4.60 ± 1.30	1.30	39.40
0.60	0.80	3.30	5.20 ± 1.30	1.90	57.80
0.80	1.07	3.30	5.90 ± 1.30	2.60	78.80
1.00	1.30	3.30	7.50 ± 1.30	4.20	127.30

Standard deviation (S.D) of extract treated segment = ± 1.30

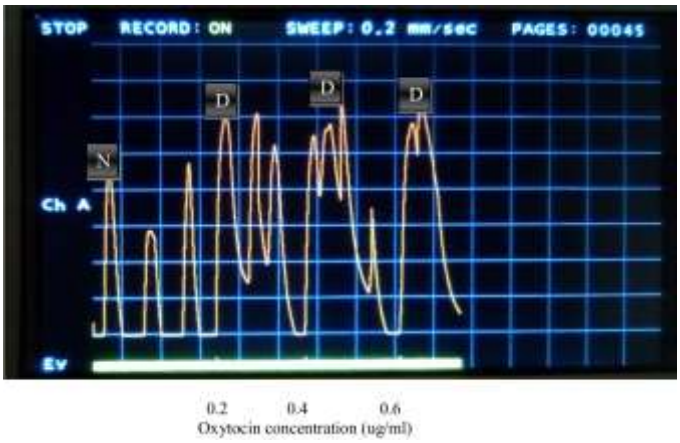

Figure 3: Oxytocin induced contraction amplitude in dose-dependent manner on albino rat uterus *in-vitro* (1 ug/ml)
Key: N= Normal contraction (without drug); D= Drug induced contraction

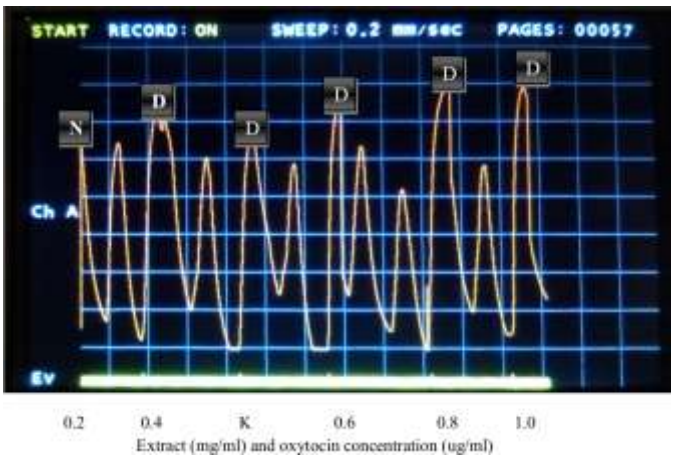

Figure 4: Methanol plant extract of *Momordica charantia* and Oxytocin (1 ug/ml) induced contraction amplitude in dose-dependent manner on albino rat uterus *in-vitro* (100 mg/ml)

Key: N= Normal contraction (without drug); D= Drug induced contraction

Table 7. Amplitude of contraction of albino rat uterus exposed to graded bath concentration of oxytocin (1 ug/ml)

Oxytocin concentration (ug/ml)	Bath concentration (ug/ml)	Normal segment (cm)	Oxytocin treated segment (cm) ± S.D	Difference in amplitude	% increase
0.20	0.0027	4.70	6.00 ± 0.53	1.30	28.00
0.40	0.0053	4.70	6.40 ± 0.53	1.70	36.20
0.60	0.0080	4.70	6.30 ± 0.53	1.60	34.00

Standard deviation (S.D) of extract treated segment = ± 0.53

Table 8. Amplitude of contraction of albino rat uterus exposed to graded bath concentrations of methanol plant extract of *Momordica charantia* (100 mg/ml) and oxytocin (ug/ml)

Ext (mg/ml) +Oxytocin (ug/ml) conc.	Bath concentration (mg/ml)	Normal segment (cm)	Extract+ oxytocin treated segment (cm) ± S.D	Difference in amplitude	% increase
0.20	0.27	5.30	6.00 ± 0.77	0.70	13.20
0.40	0.53	5.30	5.40 ± 0.77	0.10	1.90
0.60	0.80	5.30	6.10 ± 0.77	0.80	15.10
0.80	1.07	5.30	6.70 ± 0.77	1.40	26.40
1.00	1.30	5.30	7.10 ± 0.77	1.80	34.00

Standard deviation (S.D) of extract treated segment = ± 0.77

The result of the preliminary phytochemical screening of methanol extract of *Momordica charantia* revealed the presence of carbohydrates, terpenoids, cardenolides, saponins, glycosides, cardiac glycosides, and flavonoids. Similarly, the study of Patil and Patil [9] on petroleum ether, chloroform and ethanol extract of *M. charantia* reported that the ethanol extract showed positive tests for alkaloids, flavonoids, glycosides, phenols, tannins, oils and fats. The petroleum ether extract showed positive tests for steroids, oils and fats. The chloroform extract showed positive tests for steroids, alkaloids, oil and fats. In addition to this, a study by Islam *et al.* [10] showed that phytochemical screening indicated that different constituents such as saponins, tannins, triterpenes, alkaloids and flavonoids were present in the methanol plant extract of *Momordica charantia.*

The result obtained for acute toxicity (LD_{50}) of methanol seed and plant extracts of *Momordica charantia* were 288 mg/kg and 714 mg/kg respectively through the intraperitoneal route. A study by Abalaka *et al.* [11] on ethanol plant extracts of *Momordica charantia* revealed an acute toxicity (LD_{50}) value of 1200 mg/kg through the oral route. Similarly, different result was obtained by Yama *et al.* [12] who reported the acute toxicity (LD_{50}) of methanol seed extract of *Momordica charantia* via the oral route to be 4632.1 mg/ kg body weight of albino rat. The variation in LD_{50} obtained from the above studies could be a result of difference in the climatic condition of the plant, the part of the plant used and the type of solvent used in the extraction which plays a role in determining the biological activity of the plant. However, it may be deduced in all the above studies that both the plant and seed extracts are relatively safe or toxic to the rats owing to the large LD_{50} value which means that, toxicity or lethal effect could be experienced even at a low dose level of the extract.

According to Clarke and Clarke [13] Sodipo *et al.*14 any substance whose *i.p* LD_{50} in rats fall between 50 and 500 mg/kg is regarded as toxic, between 500 mg/kg but less than 1,000 mg/kg is moderately toxic and greater than 1,000 mg/kg is nontoxic. Therefore, it is implied that, the higher the LD_{50} value, the safer the extract and vice versa and the wide the range of LD_{50} denoted the safety effects of the extract. Therefore, the seed extract of *Momordica charantia* is more toxic (288 mg/kg) than the plant extract of *Momordica charantia* (714 mg/kg).

Methanolic plant and seed extract of *Momordica charantia* and oxytocin induced a dose-dependent amplitude of contraction of the isolated uterus of Wistar albino rats. The methanol plant extract showed the highest amplitude of contraction followed by the methanol seed extract; oxytocin, and the least was methanol plant extract of *Momordica charantia* which had the lowest amplitude of contraction respectively. The result obtained from the non-pregnant adult albino rats uterus in the organ bath set-up revealed that both the seed and plant methanol extracts significantly ($p<0.05$) and dose dependently induced contraction of the uterus *in-vitro* are relatively close. The methanol seed extract has shown to possess a higher affinity than methanol plant extract.

Oxytocin has a dual mechanism of action in the uterus. It binds to the myometrium oxytocin receptor to induce uterine contraction and also act on the oxytocin

receptor at the endometrium to stimulate prostaglandin release [15].

Cardiac glycosides act on cardiac smooth muscle and induce contraction by increasing the influx of intracellular calcium ions thereby increasing myosin light chain kinase and it also causes uterine smooth muscle contraction [8], so also flavonoids give effect towards uterine contraction by acting on oxytocin receptor [15]. Thus flavonoids and cardiac glycosides may have a role in stimulating uterine contraction in this study. However, the extracted chemical constituents in *Mormodica charantia* responsible for uterotonic property still remain speculative.

4. Conclusion

The result of this research has shown that the methanol extract of *Momordica charantia* has the ability to exert uterine contraction or abortifacient activity and this finding justifies its folkloric uses in traditional medicine for abortion of unwanted early pregnancy or to induce labour. The methanol extract of *Momordica charantia* contains important chemical constituents that may be responsible for its pharmacological action on the uterus.

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