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Effects of Reproductive and Contractile Potential of "Makann" a Bi-herbal Formulation in Mice

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Abstract

Spontaneous uterine contractions are strictly controlled and coordinated for the success of various reproductive functions. The aim of this study was to evaluate uterine activity of bi-herbal formulation of *G. kol*a and *C. papaya* roots in rodents. Ex-vivo BH cumulative concentrations response on spontaneous contractions, oxytocin induced contraction in the presence and absence of calcium and potassium induced contraction were determined. *In vivo* reproductive investigation in female mice and oestrogenicity in immature rats were carried out using standard methods. In uterine contractility studies, there was an increase in amplitude of spontaneous contractions with a steady decrease in the frequency. An increase in oxytocin amplitude and frequency was observed in calcium physiological salt solution (PSS) however, only amplitude was increased in oxytocin calcium-free PSS. Potassium chloride had a slight increase in amplitude. The reproductive cycle and uterothrophic assay exhibited no significant increase in relative uterine weight. The histology of the ovary, cervix and uterus showed normal cellular structural architecture. Oestrous cycle regularity and cycle number were increased in the BH treatment groups. In conclusion, an increase in female hormone as evident in oestrous

cycle response validate the use of this formulation in traditional medicine for the treatment of abnormal uterine bleeding.

Keywords: Bi-herbal formulation, Uterine activity, Reproductive cycle

INTRODUCTION

Spontaneous uterine contractions are strictly controlled and coordinated for the success of various reproductive functions. Increase in contractility or periods of relative relaxation of the non-pregnant uterus facilitates ova and sperm movement, adequate embryo implantation and expulsion of menstrual debris (Kunz and Leyendecker, 2002; Hutchings *et al.,* 2009). Insufficient, hyperactive, or asynchronous uterine contractions have been implicated in infertilities, implantation failure, endometriosis, dysmenorrhea, and abnormal uterine bleeding, affecting patient's reproduction and quality of life (Jones *et al.,* 2004; Chachamovich *et al*., 2010; Aguilar and Mitchell, 2010).

Carica papaya latex main constituents are papain and chymopapain, the teratogenic and abortifacient effects of these two enzymes are due to their ability to induce uterine contractions. Papain acts like prostaglandin and oxytocin (Adebiyi *et al*., 2007). Oxytocics are drugs that stimulate uterine smooth muscles contractions and examples are oxytocin, some prostaglandins and ergot alkaloids. They are used to stimulate or augment labor at term, control postpartum hemorrhage, correct postpartum uterine atony, induce uterine contractions after cesarean section or other uterine surgery, and therapeutic abortion (Anderson and Etches, 2007). *Carica papaya* latex extract induced a dose-dependent increase in frequency and amplitude of uterine contraction in non-gravid rat, significant increase was observed in proestrus and estrus stages compared to metestrus and diestrus stages of the oestrous cycles (Cherian, 2000). The uterine contractions induced by pure papain were not sustained for a long period and at higher concentrations because receptor proteins were affected by papain enzymatic action (Cherian, 2000). The crude papaya latex contains uterotonic property which might be a combination of enzymes, alkaloids and other substances which can induce uterine contraction (Cherian, 2000). *Carica papaya* methanol leaf extract cumulative spontaneous contraction *in vitro* experiment significantly increased baseline tone and induced spontaneous, rhythmic, myogenic contractions of a stilbesterol-pretreated, non-pregnant female and pregnant rat uterus (Odoh *et al*., 2020). Benzyl

isothiocyanate was extracted from *Carica papaya* seeds with 80 % ethanol and was suggested to be the bioactive compound responsible for irreversible tocolytic activity, myometrial and endometrial degeneration of isolated rat uterus (Adebiyi, 2004).

Flavonoid an anti-inflammatory compound was found in *Garcinia kola* seed and shown to affect ovulation negatively, which is an inflammatory process (Braide, 1993; Epsey, 1994; Akpantah *et al*., 2003). Anti-inflammatory drugs have been used to block ovulation because ovulation is an inflammatory reaction (Gaytan *et al*., 2002) this explains the reduction in the number of ova released. *Garcinia kola* seed at 200 mg/kg body weight altered oestrous cycle and partly blocked ovulation in female rats (Akpantah, *et al*., 2003). In animals pretreated with methanol extract of *Garcinia kola* seed there was no ovulation in 30 % of 100 mg/kg and no ovulation in 100 % of 200 mg/kg and 300 mg/kg (Essien and Effiong, 2014). Spontaneous uterine contractions are strictly controlled and coordinated for the success of various reproductive functions. Increase in contractility or periods of relative relaxation of the non-pregnant uterus facilitates ova and sperm movement, adequate embryo implantation and expulsion of menstrual debris (Kunz and Leyendecker, 2002; Hutchings *et al.,* 2009). Insufficient, hyperactive, or asynchronous uterine contractions have been implicated in infertilities, implantation failure, endometriosis, dysmenorrhea, and abnormal uterine bleeding, affecting patient's reproduction and quality of life (Jones *et al.,* 2004; Chachamovich *et al*., 2010; Aguilar and Mitchell, 2010).

MATERIALS AND METHODS

Plant Material and Authentication

Garcinia kola and *Carica papaya* roots were harvested from Egbekwe and Ovbiogie respectively in Ovia North East Local Government Area of Edo State in the month of November. The samples were authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State and were allotted voucher specimen numbers; *Garcinia kola* UBH-365 and *Carica papaya* UBH-C505.

Plant Extraction

The roots of the two plants were properly rinsed, cut into small pieces and dried under a shade for two weeks. The dried roots were further dried in hot air oven at 60 °C for 6 hr. before pulverizing separately into powder using a laboratory milling machine. *Garcinia kola* (100 g) and *Carica papaya* (100 g) roots were macerated separately in boiled water, absolute methanol, ethyl acetate, n-hexane. In the bi-herbal formulation, 50 g of *Garcinia kola* with 50 g of *Carica papaya* roots were combined (100 g) and was macerated in boiled water and absolute methanol. They were left at room temperature (30 °C ± 2 °C) with frequent shaking for 72 hr. They were filtered using glass funnel tightly plugged with cotton wool and the filtrates were concentrated in a hot air oven at 60 °C. They were properly labeled and kept in the refrigerator at 4 °C for use.

Experimental Animals

Albino adult female mice (20 – 30 g) were used. The animals were maintained at the Animal Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria. Ethical approval (EC/FP/021/20) was obtained from the Ethics Committee on the Use of Animals for Experimental Procedures, Faculty of Pharmacy, University of Benin. The animals were housed in well ventilated cages and fed with animal pellets (Top feed) with free access to clean water *ad libitum*. All animals were handled carefully according to the Guide for the Care and Use of Laboratory Animals (2011). All animals were weighed on S-Mettler electronic compact balance model K-500BH (max=500, d=0.01g), weight was recorded in grams. Anal temperature was taken using C-Tone digital thermometer (Mode: GF502) in degree Celsius (°C).

Preliminary Uterine Contractility Study

Spontaneous and oxytocin induced contractions of all the extracts were determined in virgin female mice uterine isolated tissue in a single concentration response (100 mg/ml) in 10 ml UgoBasile organ bath containing aerated physiological salt solution of the composition in mM: NaCl 154.0, $NaHCO_3$ 5.95, KCl 5.63, D-glucose 2.77 and $CaCl_2.2H_20$ 0.648. The aqueous extract of the 50 g of *Garcinia kola* with 50 g of *Carica papaya* roots combined (100 g), named bi-herbal root formulation of *Garcinia kola* and *Carica papaya* (BH) was used for all successive experiments.

Vaginal Lavage

Macroscopic and microscopic examination of mice vagina were carried out between 10 am and 12 noon daily. Microscopic experiment was carried out by vaginal lavage, 0.1 ml normal saline was withdrawn with a disposable micropipette to flush the vagina. This was transferred to a clean microscope slide to prepare a smear. The smear was dried in a hot air Oven at 50 °C for 5 min and stained with 0.005 $^{0}/_{0}$ Methylene blue for 3 min, the stain was washed off with distilled water and dried (McLean *et al.*, 2012). The stained smears were viewed with an S-Viewer digital Camera (14M USB2.0, UHD-14-AGC) mounted on a LABO Microscope AXL (Germany) connected to an *hp* laptop. Photomicrograph was taken with x10 and x40 objectives.

***Ex Vivo* Uterine Contractility**

Adult female mice (≈ 28-30 g) were used for this study. Before the start of experiments, vaginal lavage was taken and microscopically examined after staining with methylene blue. The animals in oestrus were euthanized by cervical dislocation under the code of ethics for humane killing of animals (Committee, 2011). The uterine horns were carefully harvested and surrounding fats and mesenteries cut off 5 mm length of uterine horn was cut and mounted in a 10 ml organ bath containing physiological salt solution (PSS) of the following composition in Moles: NaCl 154.00, $NaHCO_3$ 5.95, D-glucose 2.78, KCl 5.63, and $CaCl_2{\cdot}2H_2O_2.0_5$ maintained at 37 °C, aerated by an aerator. The cut out uterine tissues was placed under 5.0 g tension and allowed to equilibrated for 30 min or till regular contraction was obtained. The amplitude and frequency of contractions generated from the longitudinal muscle layers of the uterine tissue were recorded using a transducer connected to a digital recorder and a computer. The effect of cumulative BH extract of 10, 100 and 1000 mg/ml on uterine contractility were recorded, (n = 5).

BH Extract on Spontaneous Uterine Contraction

The cumulative effect of 10, 100 and 1000 mg/ml BH extract on spontaneous uterine contraction were studied using the method of Bafor *et al.* (2010). The concentration–response relationships was obtained with a contact time of 5 min per concentration. At the end of each experiment, the tissue was washed 3 times with PSS and left to recover (n = 5).

Oxytocin and Potassium Induced Contractions

The activities of BH extract in oxytocin-induced uterine contraction (1 IU) Rotex Medica (Germany) and high KCl-induced uterine contraction (100 mM) were investigated. BH extract was also assessed in the absence of extracellular calcium using physiological salts solution in which $CaCl_2$ had been omitted and 1 mmol/L Ethylenediamine tetraacetic acid (EDTA) added (Bafor *et al*., 2010).

Female Reproductive Cycle

The reproductive cycles of virgin female mice (12 weeks) were first determined using microscopic examination of stained vaginal lavage. The vaginal lavage was used to determine mice oestrous cycle phases (proestrus, oestrus, metoestrus and dioestrus) according to cell types; leucocyte, nucleated epithelial cell and cornified epithelial cell (Champlin *et al.,* 1973). Animals in the same oestrous phase were grouped together and assigned into 6 groups of 5 animals per group. Group 1 was control and was given 10 ml/kg of distilled water; groups 2, 3 and 4 received 1, 10 and 100 mg/kg of BH extract respectively. Group 5 was administered oestradiol 10 mg/kg and group 6 was given progesterone 10 mg/kg. All administrations were done orally for 7 days. Vaginal lavage, weight and temperature assessment continued all through the period of administration. The animals were euthanized 24 hr after the last dose of administration by chloroform inhalation. The uterus, with the cervix and ovary intact, was harvested, weighed, and fixed in Bouins fluid for histopathological examination (Champlin *et al*., 1973; Elvis-Offiah and Bafor, 2014).

RESULTS

Percentage yield of extracts

The different solvents used for extraction had effect on the percentage yield of *Garcinia kola* and *Carica papaya* roots individually and when combined. *Carica papaya* root aqueous extract had the highest yield of 33.15 % followed by aqueous extract of the bi-herbal formulation 20.41 %; and *Garcinia kola* root methanol extract had higher yield (9.83 %) compared to the bi-herbal methanol extract (5.68 %). *Garcinia kola* root lowest yield was in hexane extract (0.39 %) while *Carica papaya* lowest yield was in ethyl acetate extract (0.21 %) as shown in Table 1.

Table 1: Effect of solvent on extraction and percentage yield

PLANT	SOLVENT	EXTRACT APPERANCE	% YIELD
Garcinia kola root	Distilled water	Light brown, sticky	3.38
	Methanol	Light brown, gum	9.83
	ethyl acetate	Light brown, gum	9.29
	Hexane	Light brown, oil	0.39
Carica papaya root	Distilled water	Dark brown, thick	33.15
	Methanol	Brown, slightly thick	5.38
	ethyl acetate	Brown, slightly powdery	0.21
	Hexane	Brown, oil	0.28
Garcinia kola and *Carica papaya* combination	Distilled water	Dark brown, thick	20.41
	Methanol	Brown, slightly thick	5.68

Ex Vivo Uterine Contractility

Spontaneous uterine contractions of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) cumulative concentration revealed an increase in amplitude and a gradual reduction in frequency at lower concentration of 10 – 100 mg/ml but reduced amplitude and frequency were observed at the highest concentration of BH extract 1000 mg/ml (Figures 1 and 2).

There was increased the frequency and amplitude of 1IU oxytocin contraction in BH extract 12.21µg/ml final organ bath concentration in calcium physiological salt solution, as depicted in Figures 3 and Figure 4. In calcium free physiological salt solution BH extract 12.21µg/mL final organ bath concentration increased amplitude but decreased the frequency of 1IU oxytocin contraction (Figures 5 and Figure 6).

Figure 7 showed marginal increase in amplitude in potassium induced contraction in 12.21 µg/ml final organ bath concentration.

Reproductive/Oestrous Cycle of Mice

In Figures8 and Figure 9, aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) 1, 10, 100 mg/kg, oestradiol 10 mg/kg and progesterone 10 mg/kg, the body weight and temperature were not statistically different when compared with the control.

Regular oestrous cycle sequence of oestrus, metestrus, diestrus, proestrus was observed in photomicrographs of treated groups as depicted (Plate 1). In 1mg/kg 100 $^{0}/_{0}$, 10 and 100 mg/kg 80 $^{0}/_{0}$ of treated mice recorded regular oestrous cycles but mice in oestradiol 10 mg/kg and progesterone 10 mg/kg had 0 $^{0}/_{0}$ regular oestrous cycles compared with the control of 60 $^{0}/_{0}$ regularity as observed in Figure 10. Figure 11-12 showed the oestrous cycle numbers in the different treatment groups; the highest oestrous cycle number was in 1mg/kg treatment group, followed by 10 and 100 mg/kg, oestradiol 10 mg/kg and non in progesterone 10 mg/kg.

Histology photomicrographs of uterus in BH extract treatment groups revealed normal and small proliferating endometrial glands supported by plump endometrial stroma. Proliferative endometrial glands lined by secretory epithelium supported by plump stroma was revealed in Oestradiol group. Progesterone group revealed endometrial glands lined by thick epithelial cells supported by plump endometrial stroma compared with the control with normal endometrial glands in proliferative phase supported by plump endometrial stroma (Plate 2 - 4).

Figure 1a: Organ bath tracing of cumulative spontaneous contraction of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) in non-gravid mice uteri *ex vivo*

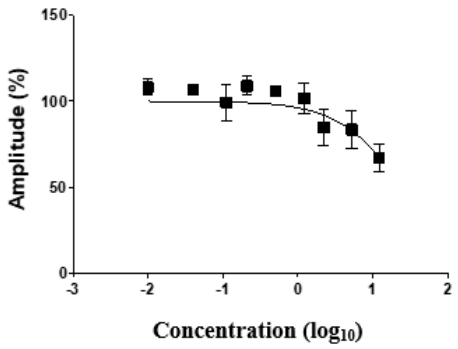

Figure 1b: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on spontaneous contraction amplitude in non-gravid mice uteri *ex vivo*

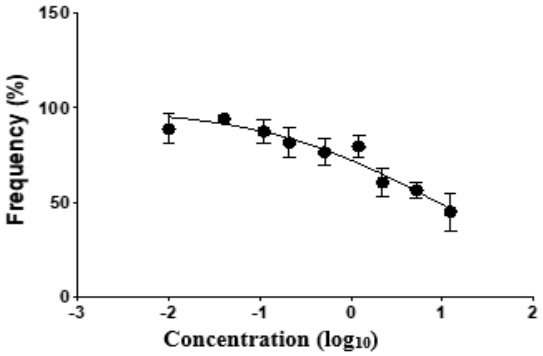

Figure 2: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the frequency of spontaneous of non-gravid mice uteri contraction *ex vivo*

Figure 3a: Organ bath tracing of 1IU Oxytocin (OT) –induced contraction of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) in non-gravid mouse uterus *ex vivo*

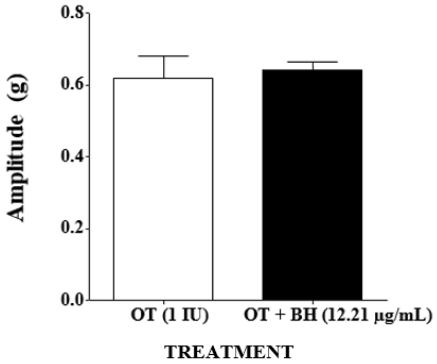

Figure 3b: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the amplitude of 1IU Oxytocin (OT) –induced contraction in non-gravid mouse uterus *ex vivo*

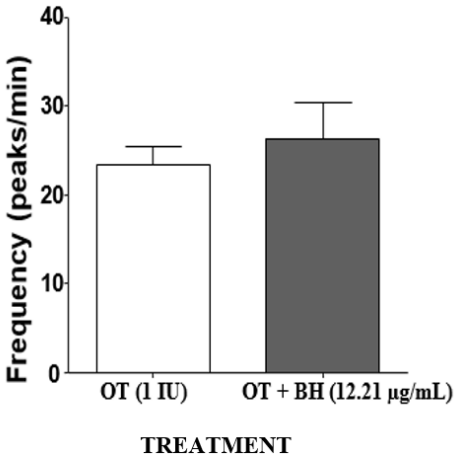

Figure 4: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the frequency of 1IU Oxytocin (OT) – induced contraction in non-gravid mouse uterus *ex vivo*

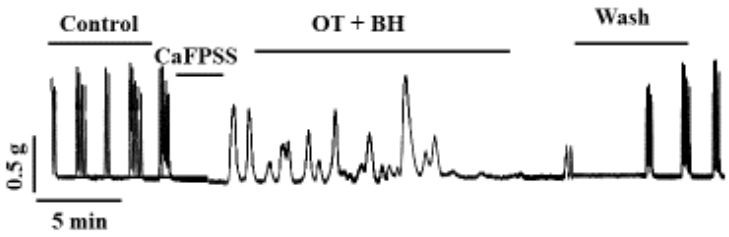

Figure 5a: Organ bath tracing of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the amplitude of 1 IU Oxytocin-induced contraction in Calcium free physiological salt solution (PSS) in non-gravid mouse uterus *ex vivo*.

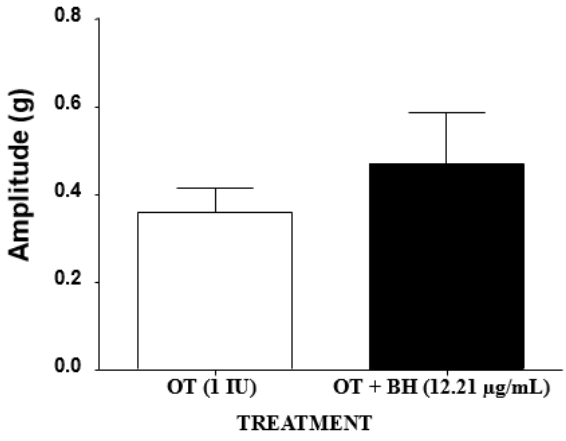

Figure 5b: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the amplitude of 1 IU Oxytocin-induced contraction in Calcium free physiological salt solution (PSS) in non-gravid mouse uterus *ex vivo*.

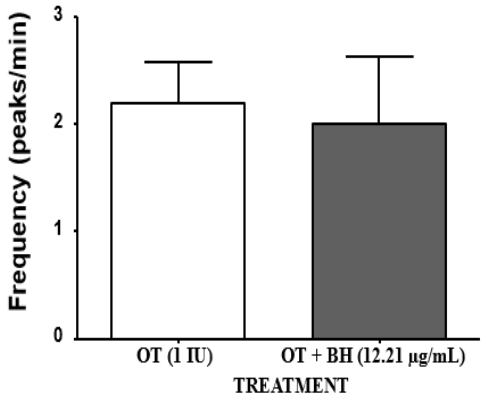

Figure 6: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the frequency of 1IU Oxytocin-induced contraction in Calcium free physiological salt solution (PSS) in non-gravid mouse uterus *ex vivo*

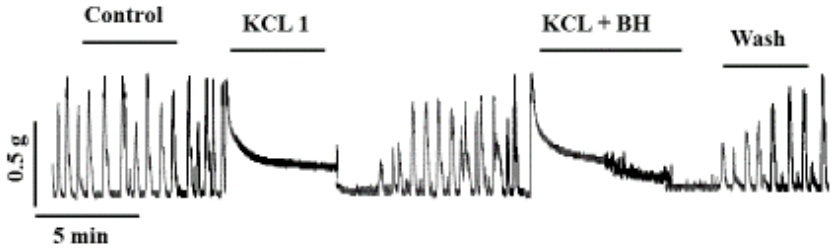

Figure 7a: Organ bath tracing of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the amplitude of high Potassium chloride (KCl)-induced contraction in non-gravid mouse uterus *ex vivo*

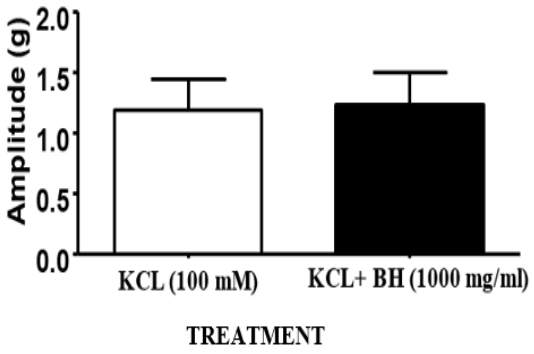

Figure 7b: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the amplitude of high Potassium chloride (KCl)-induced contraction in non-gravid mouse uterus *ex vivo*

Cervix in 1 mg/kg treatment group revealed normal ectocervical epithelium supported by normal cervical stroma, 10 mg/kg group showed normal ectocervical epithelium with luminal mucus supported by normal cervical stroma, 100 mg/kg group, normal ectocervical epithelium supported by thin myxoid stroma. Oestradiol thickened ectocervical squamous epithelium supported by plump ectocervical stroma and Progesterone, atrophied ectocervical squamous epithelium supported by thin myxoid stroma compared with the control with normal ectocervical epithelium supported by normal cervical stroma (Plate 21).

In all BH extract treatment groups, the ovaries revealed normal follicles in different stages of maturation (graffian follicle and tertiary follicle), in oestradiol group folicles in different stages of maturation and progesterone, normal tertiary follicle and were all supported by normal ovarian stroma were observed (Plate 22).

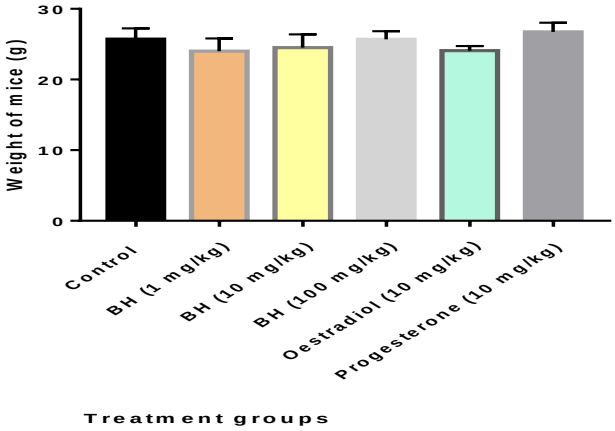

Figure 8: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on body weight in female reproductive cycle in mice

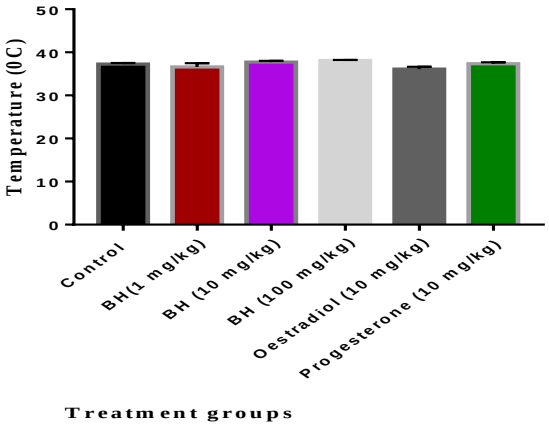

Figure 9: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on body temperature in female reproductive cycle in mice

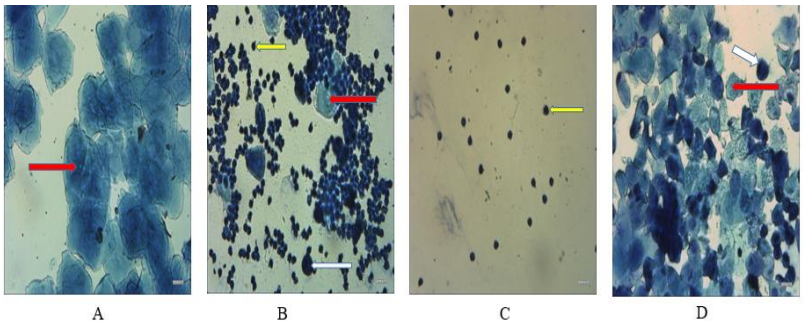

Plate 1: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on oestrous cycle stages of a mouse vaginal smear (stain methylene blue, X40 objective).

Key: A – oestrus, B – metestrus, C – diestrus, D – proestrus. Red arrow– cornified epithelial cell, White arrow– neucleated epithelial cell, Yellow arrow—leucocyte.

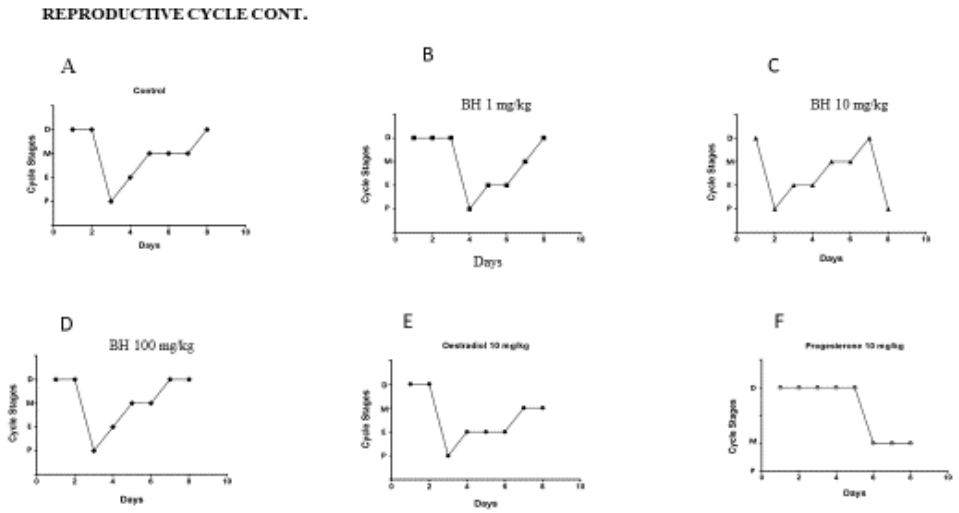

Figure 8. Oestrous cycle staging of treatment groups, 10 mg/kg Oestradiol group stayed longest in oestrous phase while 10 mg/kg Progesterone stayed longest in diestrous phase, n = 5.

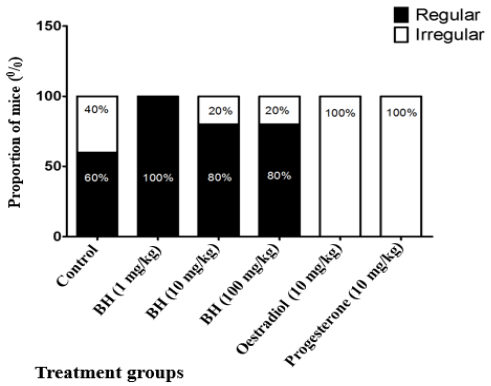

Figure 10: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on oestrous cycling regularity in female mice oestrous cycling

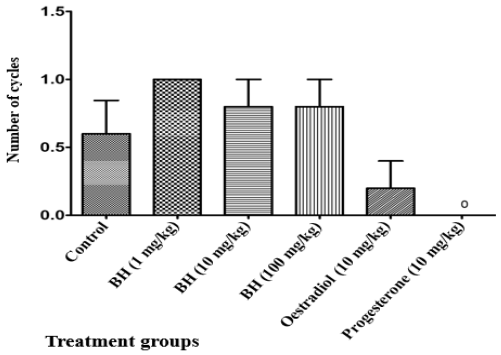

Figure 11: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the number of oestrous cycles in female mice oestrous cycling.

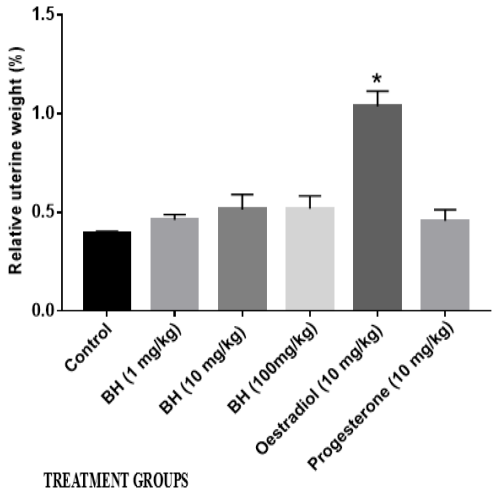

Figure 12: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative uterine weight in female mice reproductive study.

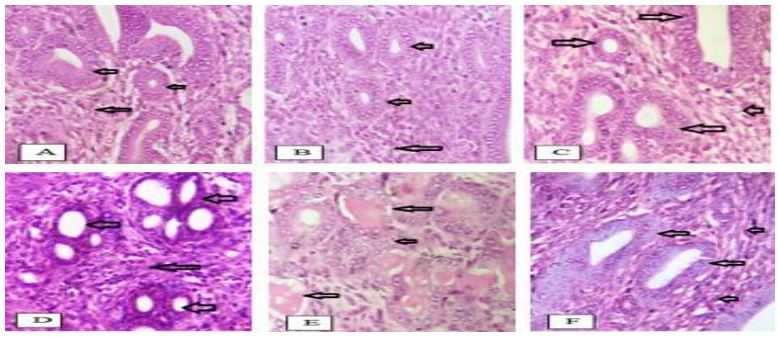

Plate 2: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the histopathological structure of the mice uterus in reproductive cycle (stain H & E, X40 objective).

A – CONTROL reveals normal endometrial glands in proliferative phase (short arrow) supported by plump endometrial stroma (long arrow).

B – BH extract 1 mg/kg reveals normal endometrial glands (short arrow) supported by plump endometrial stroma (long arrow).

C – BH extract 10 mg/kg reveals small proliferating endometrial glands (short arrow) supported by plump endometrial stroma (long arrow).

D – BH extract 100 mg/kg reveals small proliferating endometrial glands (short arrow) supported by plump endometrial stroma (long arrow).

E – Estrogen 10 mg/kg reveals proliferative endometrial glands lined by secretory epithelium (long arrow) and supported by plump stroma (short arrow).

F – Progesterone 10mg/kg reveals endometrial glands lined by thick epithelial cells (long arrow) supported by plump endometrial stroma (short arrow).

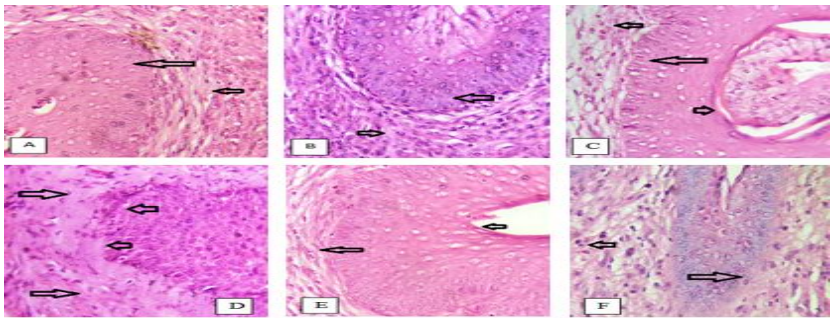

Plate 3: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the histopathological structure of the mice cervix in reproductive cycle (stain H & E, X40 objective).

A – CONTROL reveals normal ectocervice (long arrow) and normal cervical stroma (short arrow).

B – BH extract 1 mg/kg reveals normal ectocervical epithelium (long arrow) supported by normal cervical stroma (short arrow).

C – BH extract 10 mg/kg Cervix reveals normal ectocervical epithelium with luminal mucus (long arrow) supported by normal cervical stroma (short arrow).

D – BH extract 100 mg/kg reveals normal ectocervical epithelium (short arrow) supported by thin myxoid stroma (long arrow).

E – Oestradiol 10 mg/kg reveals a thickened ectocervical squamous epithelium (short arrow) supported by plump ectocervical stroma (long arrow).

F – Progesterone 10mg/kg reveals atrophied ectocervical squamous epithelium (long arrow) supported by thin myxoid stroma (short arrow).

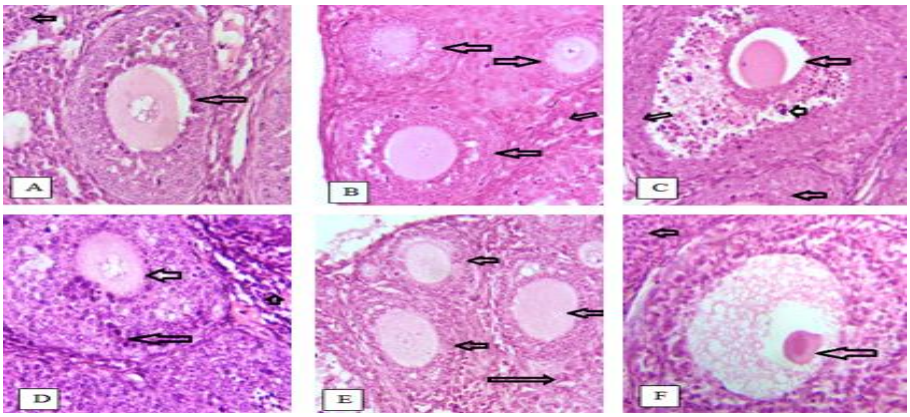

Plate 4: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH on the histopathological structure of the mice ovary in reproductive cycle (stain H & E, X40 objective).

A – CONTROL reveals normal secondary follicle (long arrow) supported by normal ovarian stroma (short arrow).

B – BH extract 1 mg/kg reveals normal follicles in different stages of maturation (long arrow) supported by normal ovarian stroma (short arrow).

C – BH extract 10 mg/kg reveal normal graffian follicle (long arrow) with an antrum (arrowhead) and tertiary follicle (short arrow) supported by normal stroma.

D –BH extract 100 mg/kg reveals normal tertiary follicle (long arrow) with centrally placed oocyte (short arrow) supported by normal ovarian stroma (arrowhead).

E – Oestradiol 10 mg/kg reveals folicles in different stages of maturation (short arrow) supported by normal ovarian stroma (long arrow).

F – Progesterone 10mg/kg reveals normal tertiary follicle (long arrow) supported by normal ovarian stroma (short arrow).

DISCUSSION

Plants are endowed with bio-compounds, which are secondary metabolites with therapeutic and prophylactic properties. BH extract increased oxytocin induced contraction, however, in the absence of calcium, frequency was reduced. An increase in amplitude of BH extract

in potassium induced contraction was also recorded. These results showed BH extract causes an influx of calcium from the extracellular space into the intracellular cell through the L-type voltage operated calcium channel, mobilize calcium from the internal calcium stores of the endothelia reticulum resulting in uterine tissue contractions this is in agreement with the report of Streb *et al.* (1983) on the role of cellular calcium in tissue contractility. Depolarized myocytes membrane, opens L-type voltage operated calcium channel thereby increasing intracellular calcium ions (Sukwan *et al.,* 2014). Four ions of calcium bind to one calmodulin, forming a calcium-calmodulin complex (CaCC). Calcium-calmodulin complex activates myosin light chain kinase, which catalysis the phosphorylation of myosin light chain generating force that contracts myometrial smooth muscles. Contraction of the uterus is important in control of bleeding in menstrual flow and after child birth. One of constituents of BH extract is *Carica papaya* root and its latex has been reported to induce uterine contraction (Odoh, 2020). Reduction in uterine contraction at higher concentration is corroborated by the findings of Adebiyi (2004). It was observed in the oestrous cycle results that BH extract at a dose of 1 mg/kg which was the lowest dose of treatment, recorded the highest number of oestrous cycles with oestrous cycles regularity of 100 % however, the number of oestrous cycles and oestrous cycle regularity at 10 and 100 mg/kg were not different (Figures 1-12). The normal oestrous cyclesequence of oestrus, metoestrus, dioestrus and proestrus was observed in all treated groups. *Carica papaya* and *Garcinia kola* seeds have been reported to disrupt oestrous cycle regularity and ovulation (Akpantah *et al.,* 2003; Raji *et al.,* 2005). However, the aqueous extract of the composite formulation of *Carica papaya* and *Garcinia kola* roots in female mice at the studied doses and duration of treatment had positive influence on female reproductive cycle and mayenhance female fertility. The disparity of this result from those previous reported could bebecause of the plants part used and also, the combination of the 2 plants roots would have ameliorated their side effects on oestrous cycle regularity and ovulation.

BH extract and progesterone treated groups did not significantly increase the relative uterine weight of mice. However, oestradiol treated group significantly increased the relative uterine weight. The increase in relative uterine weight in BH extract 10 and 100 mg/kg doses, though not significant was more than the increase observed in 1 mg/kg. The increased in uterine weight did not induce a corresponding increase in body weight as oestradiol 10 mg/kg with significant increase in uterine weight had the lowest body weight

compared to all other treated groups. This result suggests the bi-herbal formulation having mild increase of uterine activities that are not oestrogenic as shown in Figure 6. Histopathology structure of the uterus in reproductive study revealed normal to small proliferating endometrial glands supported by plump endometrial stroma. The cervix showed normal ectocervice and cervical stroma and ovary had follicles at different stages of development supported by normal ovarian stroma. The histology result of BH extract could be said to have a positive effect on uterine activities and female reproductive hormonal balance. However results differ from the report of Iranloya and Owokunle (2008) on the effect of *Garcinia kola* seed extract on reproductive cycle and pregnancy in female rats, reported a reduction of oestrus, metestrus, ovulation, endometrial activities, absence of follicular maturation, inactive endometrial gland, fallopian tubes inflammation and increase in proestrus and dioestrus. Flavonoid found in *Garcinia kola* seed have been reported to prevent ovulation (Akpantah et al., 2003). The absence of these negative reproductive cycle effect observed in BH extract could be as a result of either the difference in plant part used or the composite bi-herbal formulation with ameliorative effect of the herbal-herbal formulation. Uterotrophic assay of different doses of BH extract 10, 100 and 1000 mg/kg and oestradiol 10 mg/kg in immature rats of 18 days old for 3 days suggested that BH extract did not negatively affect the appetite, digestion or absorption of nutrients as there was daily increase of food consumed in all groups. The significant increase in body weight of control group and BH 10 mg/kg treated group corresponded to the quantity of feed consumed. However, there was no significant increase in body weight of oestradiol treated group but it induced significant increase in relative uterine weight and oestradiol blood level. BH extract treated groups induced a no significant dose dependent increase in oestradiol blood level but this did not elicit corresponding increase in uterine weight as the indicators of oestrogenic effect; imbition of fluid into the uterus and proliferation of uterine tissue were not observed in BH extract treated groups. Oestrogen is a female hormone that is released mainly from the ovary in the proliferative phase of the reproductive cycle with resultant endometrial proliferative growth (Rhoades *et al.,* 2013). The oestrogenic property of Garcinia kola seed was observed by Essien and Effiong (2014) and non- oestrogenic effect of *Carica papaya* seed was reported by Raji *et al.* (2005), the absence of oestrogenic effect in our study could be as a result of *Carica papaya* ameliorating the *Garcinia kola* oestrogenic property.

Photomicrographs of haematoxylin and eosin stained tissues of BH extract and oestradiol treated groups showed normal uterine lining with small glands, the size of the endometrial glands could be because of the immature rats used in this work that had not reached reproductive age. The cervix had normal ectocervical, endocervical and cervical stroma with ovary showing follicles in normal sequential maturation. BH extract did not alter the normal cellular structural architecture in growing immature female rat (Brielmann et al., 2006; Chen et al., 2011). Imperialine-3β-D-glucoside and other alkaloids have been demonstrated to induce smooth muscles contractions (Sukwan, et al., 2014; Liu et al., 2018). Alkaloids from the latex of *Carica papaya* have been reported by Cherian (2000) to induce uterine contractions while *Garcinia kola* seeds alkaloid was observed to inhibit uterine contractions. *Carica papaya* different plant parts have several secondary metabolites with therapeutic, prophylactic efficacies and had been reported to possess fertility, anti-fertility, anti- inflammatory, antioxidant, antimicrobial, anticancer, anti-hypertension, and anti-diabetic and other pharmacological properties (Vishwanath *et al.*, 2016).

CONCLUSION

In conclusion, sowedthe theapeutic effects of abnormal uterine bleeding due to protracted or heavy bleeding is given scientific backing and validated by the results of this research work. BH extract increased oestrous cycle regularity and therefore enhance fertility in female mice.

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