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Research Article

Contragestive Activity of *Citrullus Colocynthis* (L.) Schrad (Bitter Apple) Extract is Via Oestrogen-Progesterone Suppression

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ABSTRACT

Citrullus colocynthis fruit is commonly used in traditional medicine as a therapy for numerous diseases, including antifertility and uterine cleansing. This study investigated the contragestive activity of *Citrullus colocynthis* fruit in pregnant Wistar rats. Pregnant rats were randomized into six groups (n=5 rats/group): Control (received olive oil only), MIFP (1.84 mg/kg mifepristone standard drug), while other groups were administered with 100, 200, 400, and 800 mg/kg ethanol extract of *Citrullus colocynthis* (EECC) from day 6th through 14th. Thereafter, the abortifacient parameters and reproductive hormone levels were evaluated. The result obtained during the *in vitro* analysis of EECC using GC-FID revealed the presence of bioactive constituents like cucurbitacin B and gallic acid. However, treatment with EECC, p.o for 9 days, led to a dose-dependent decrease ($p < 0.05$) in foetus survival ratio at 81.48%, 66.66%, 18.75%, and 0%, with increase in EECC concentration. Likewise, the influence of the treatment resulted in a dose-dependent elevation in abortion, pre- and post-implantation losses, and resorptive index. In contrast, a decrease in implantation index and *Corpora lutea* was recorded. Mifepristone and 800 mg/kg EECC showed 100% post-implantation loss. Moreover, the group receiving 800 mg/kg EECC have comparable abortifacient effects with the mifepristone-treated group. Furthermore, a dose-dependent reduction in progesterone, oestrogen, follicle-stimulating hormone, luteinizing hormone, and prolactin in the serum was recorded in EECC-treated rats, hence, supporting its use in folkloric medicine as a contragestive. Hence, cucurbitacin B and gallic acid present in EECC extract could be responsible for contragestive potential.

Keywords: *Contragestive; Abortifacient; Citrullus Colocynthis; Mifepristone; Progesterone*

INTRODUCTION

Contragestives are substances that can alter the pregnancy process and reproductive hormones after implantation, at the onset of pregnancy, and during early pregnancy. Choucroun (2024) documented that contragestives are recognized as agent that induces abortion through altering, blocking, and interfering with the female reproductive hormones, thereby hindering the intrauterine development of the foetus. Moreover, the use of appropriate contragestive methods will help in the prevention of implantation, unintended pregnancy and facilitate efficient family planning. In 2020, Bearak and colleagues documented that approximately four out of ten of all pregnancies end in induced abortion in low-income countries while nearly half of all abortions are unsafe (Haddad and Nour, 2009), and majority take place in Africa and Latin American countries (Ganatra et al., 2017). Therefore, due to the unnecessary risks, diseases, long-term complications, and even death arising from the unsafe abortion, the World Health Organization in 2019

documented that some herbal preparations and herbal products have been widely used as traditional herbal medicines, mostly by women in developing countries (Hall et al., 2011). Hence, the need to examine the need of herbal medicine as contragestive.

Though, in 2010, Yakubu et al reported that abortion is mostly done by the use of steroidal contraceptives such as mifepristone or/and misoprostol, prostaglandins, anti-progestins, and surgical intervention. They also highlighted several adverse effects associated with these synthetic drugs (Yakubu et al., 2010). There is a wide range of data available over the years depicting the potentials of medicinal plants as an abortifacient from the ethnobotanical survey, but there is a need to explore it contragestive efficacy. Although studies have been carried out to validate the use of some plants, such as *Anthocephalus cadamba* (Roxb.) Miq. (Shaikh et al., 2015), Musa rosacea Jacq. (Srikanth et al., 2013), *Senna alata* (L.) Roxb. (Yakubu et al., 2010) and *Bambusa vulgaris* Schrad. (Yakubu and Bukoye, 2009) as an abortifacient, *Citrullus colocynthis* (L.) Schrad. belongs to the Cucurbitaceae plants, also known as bitter apple (English), bara (Yoruba), kabawa (Hausa), and ogbe (Ibo) in Nigeria.

Citrullus colocynthis (L.) Schrad. belongs to the Cucurbitaceae plants, also known as bitter apple (English), bara (Yoruba), *kabawa* (Hausa), and ogbe (Ibo) in Nigeria. There are several reports on the medicinal attributes of all the parts of the plant *Citrullus colocynthis* which involves their application in the management of constipation, edema, bacterial infections, cancer (Abdulridha et al., 2020), diabetes (Gurudeeban et al., 2010), antimicrobial and anti-inflammatory effects (Marzouk et al., 2011) as well as their antioxidant and free radical scavenging potentials have been documented (Sireesha and Raghunandan, 2018). Local folkloric medicine claimed that *Citrullus colocynthis* is normally applied for easy delivery, uterus washing, and abortion (Yadav et al., 2006). Qazan et al. (2007) also document the short- and long-term effect of *Citrullus colocynthis* effect reproductive system and female fertility. The teratogenic effects of isolated bioactive principles from Citrullus colocynthis in albino was also studied by lgerwi et al. (2013). Hence, there is no information regarding the contragestive effect of *Citrullus colocynthis*. In this study the contragestive potentials and the mechanism of action explored by *Citrullus colocynthis* in pregnant Wistar rat was examined.

MATERIALS AND METHODS

Plant materials

Citrullus colocynthis fruits were obtained from the Nigeria Stored Product Research Institute, Ilorin, and authentication was done at the Botany Department by Mr Bolu Ajayi a senior Laboratory Superintendent, University of Ilorin, Nigeria, and assigned specimen number UILH 001/1160.

Drugs and animals

Progesterone, oestrogen, prolactin, follicle-stimulating hormone, and Luteinising hormone was procured from Monobind Inc., USA. Mifepristone was purchased from West Coast Pharmaceutical Ltd. Gujarat India. Folin-Ciocalteu, diethyl ether, Sodium nitrite ($NaNO_2$), sodium chloride, sodium hydroxide, aluminum trichloride (AlCl3), anhydrous sodium carbonate (Na_2CO_3), ammonia solution, ferric chloride ($FeCl_2$), absolute ethanol, and mono- and di-basic sodium phosphate were procured from Merck and Sigma-Aldrich, Gauteng, South Africa. Other reagents used were of diagnostic grade and prepared in accordance with the standard procedures.

Laboratory animals

The female albino rats used were weighing 165-175g, and the matured male albino rats used were weighing between 179-190g; rats were procured from the Institute for Advanced and Medical Research (IAMRAT), University of Ibadan, Oyo State. The animals were acclimatized in Standard laboratory conditions of 25 ^oC, 12 hours light and dark cycle, with unrestricted access to tap water *ad libitum* and rat chow for two weeks before they were paired. The experiment was conducted after obtaining Ilorin University Ethical Review Committee approval on the use and care of experimental animals with ref UIL/UERC/LSC/2015/023.

Extract preparation

Yakubu *et al*. (2005) methodology was adopted in the preparation and extraction of the extract derived from *Citrullus colocythis* with little modification. Briefly, the fruits free of the seeds were dried at 40°C in the oven for 72 hrs to obtain a constant weight and then crushed using Euro Premium Domestic Mixer Grinder. 200g of the crushed sample was then macerated with 1L of ethanol and shaken continuously for 72 hrs at 25 °C. Thereafter, it was filtered using Whatman No. 1 filter paper. The pure filtrate obtained was subsequently concentrated under pressure by a rotary evaporator at 40 °C to obtain a brownish slurry extract of *Citrullus colocythis* having a final weight of 19.30g. The extract was then stored in an amber bottle at -20 °C until further use.

Secondary metabolites analysis

Quantitative determination of bioactive components such as alkaloids (Harborne, 1998), tannins (Amadi et al., 2004), terpenes (Ghorai et al. 2012), saponins (Brunner, 1984), , phenolics using Folin Ciocalteu protocol (Samtha et al., 2012), flavonoids using colorimetric method (Babouchi et al., 2019), anthraquinones (Kurkin et al., 2017), and glycosides (Harborne, 1998) were performed on the extract. The EECC was further subjected to GC-FID analysis to determine the bioactives presents in the crude extract (Fig. 1). In brief, The GC-FID analysis of the plant extract was carried out on a Hewlett Packard Series II using a programmed temperature, 220 ^oC for the injector, 250 ^oC for the oven and 280 ^oC for the detector while the holding time

was 2 mins. Hydrogen gas serves as the combusting gas and Nitrogen as the carrier gas. 20 ml of the extract was weighed into a glass separating funnel with a stopper and cork and 20 ml of hexane was added. The mixture was shaken vigorously for 30 min while occasionally releasing the cork to let out built pressure within the funnel. After which the hexane layer is collected into a glass beaker and allowed to evaporate in air. The concentrated extract is transferred to a sample vault, then to refrigerator waiting for analysis. 1 ml of the standard hydrocarbon is injected into the gas chromatography (GC) and a standard chromatogram generated. This standard chromatogram is used to standardize the GC in preparation of the sample analysis. Thereafter, 1 ml of the extract was injected into the GC and a corresponding chromatogram generated. The chromatograms of both the standard and test sample were then compared with respect to the concentration of standard.

Abortifacient study

The methodology of Ochiogu et al. (2006) was applied for the abortifacient which involved pairing of female and male albino rats overnight in cages and maintained on rat chow and water in ratio 2:1. The vaginal smear was collected by carefully inserting a cotton-tipped swap moistened with normal saline and rolled on a labeled clean glass slide to detect the presence of protein coagulates under the microscope. The presence of the protein coagulates signifies the onset of pregnancy, which is day zero. The pregnant rats were thereafter sorted into six groups with five rats each.

On days 6th to 14th, the rats in Control group received (p.o.) 0.5ml of olive oil daily, MIFP group received 1.84 mgkg-1 mifepristone, while the remaining three groups were administered (p.o.) 100, 200, 400, and 800 mgkg-1 ethanol extract of *Citrullus colocynthis* fruits, respectively. The rats were euthanized using light ether 24 hours after the last treatment on day 14th. Thereafter, the number of live and dead foetuses, % survival ratio, number of *corpora lutea*, number of rats aborted, % rats aborted, number of implantations, and resorption site and index, implantation index, and pre- and post-implantation losses were computed. These indices were calculated using the formulas listed in the appendices.

Quantitative determination of hormones

The hormones were quantitatively determined using Accu-Bind enzyme-linked immunosorbent assay kits by the principle of antigen-antibodies reaction in ELISA plate reader.

Statistical analysis

The data obtained were expressed as mean ± SEM. Statistical group analysis was done with GraphPad Prism 5. Mean values of variables among the groups were compared with One-way ANOVA, while Bonferroni's test was used for post hoc analysis, and were considered significant at $p< 0.05$.

RESULTS

Quantitative phytoconstituent determination of EECC

The quantitative screening performed on EECC revealed that alkaloids, tannins, phlobatannins, flavonoids, glycosides, phenolics, steroids, saponins, and terpenes were present in different concentrations, but anthraquinone, cardenolids, and chalcones were not detected (Table 1). Though alkaloids (0.52%) seem to be the most abundant followed by phenolics (0.45%) Also, analysis of the EECC by GC-FID revealed spinasterol, isovitexin, corilagin, catechin, cucurbitacin E, B, and S, quercetin, gallocatechin, myricetin, gallic acids, Cucurbitacin S, Gastrodin, Colocynthoside B, 3.0 Cafeoylquinate, Colocynthoside A were also present at varying concentrations and percentage abundance as depicted by Table 2.

Table 1. Secondary Metabolites Constituents of Ethanol Extract of *Citrullus Colocynthis*

Parameters	Concentration (%)
Alkaloids	0.52±0.03
Saponins	0.14±0.00
Tannins	0.03±0.00
Phlobatannins	0.02±0.01
Glycosides	0.12±0.00
Phenolics	0.45±0.01
Anthraquinnones	Not detected
Cardenolids	Not detected
Steroids	0.19 ± 0.02
Terpenes	0.01 ±0.00
Flavonoids	0.12±0.01
Chalcones	Not detected

n= means ± SEM (n=3)

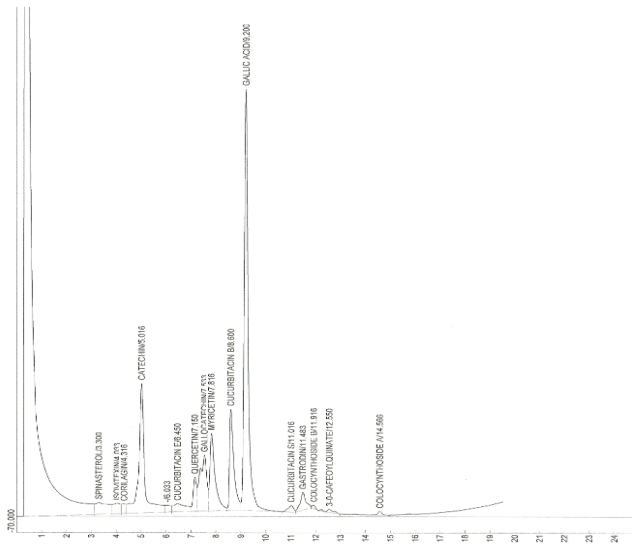

Figure. 1. Chromatogram of the different bioactive compounds of EECC as analyzed by GC-FID

Table 2. Constituents of EECC in Concentration and % Abundance as Analyze by GC-FID

Component	Conc. (mg/g)	% abundance
Spinastrol	1.28	3.27
Isovitexin	0.67	1.71
Corilagin	0.36	0.92
Catechin	10.04	25.67
Cucurbitacin E	0.87	2.22
Quercetin	0.98	2.51
Gallo catechin	3.13	8.00
Myricetin	3.41	8.72
Cucurbitacin B	3.65	9.33
Gallic acid	13.48	34.47
Cucurbitacin S	0.03	0.08
Gastrodin	0.84	2.15
Colocynthoside B	0.10	0.26
3.0 Cafeoylquinate	0.16	0.41
Colocynthoside A	0.11	0.28

Effect of EECC treatment on maternal body weight gain, number of live foetuses, and weight of the foetus

The administration of 100-400 mgkg-1 EECC led to an increase in maternal body weight gain as depicted by final body weight and percentage body weight gain in comparison with mifepristone-treated (Figure. 2A & Table 3). However, the treatment containing 800 mgkg-1 of EECC and mifepristone treatment led to reduced final maternal body weight and percentage weight gain in comparison with the control (Figure 2A & Table 3). Besides, EECC treatment caused a reduction in live foetuses with a corresponding increase in the dead foetuses when compared with control (Figure 2B &C). EECC treatment (100-400 mgkg-1) led to an increase in the number of live foetuses when compared with MIFP. However, 800 mgkg-1 EECC treatment had a comparable effect with MIFP on the number of live foetuses (Figure 2B). Furthermore, EECC treatment (100 & 200 mgkg-1) did not alter the bodyweight of the foetuses when compared with the control. But 400 mgkg-1 EECC treatment resulted in reduced body weight of foetal in comparison with control (Fig 2D). Interestingly, the 800 mgkg-1 EECC-treated and mifepristone-treated groups did not have any live foetuses to weigh. On the other hand, EECC treatment (100-400 mgkg-1) led to an increase in foetal body weight in comparison with the mifepristone-treated (Figure. 2D).

Effect of EECC treatment on survival ratio and number of aborted foetuses

There was a dose-dependent reduction in percentage survival ratio as shown by the calculated value of 81.48, 66.66 and 18.75 % for 100, 200 and 400 $mgkg^{-1}$ EECC, 0 % for the mifepristone and 800 $mgkg^{-1}$ EECC-treated respectively in comparison with the control that has 100 % survival ratio. Meanwhile groups treated with mifepristone and 800 $mgkg^{-1}$ EECC, there was 100 % abortion, whereas 20, 40, and 80 % abortion was observed in 100, 200 and 400 $mgkg^{-1}$ EECC-treated pregnant rats in comparison with the control (Table 3).

Effect of EECC treatment on abortion and survival ratio in pregnant rats

There was an increase in number and percentage of aborted foetuses in a dose-dependent manner while a decrease in survival ratio in EECC-treated rats when compared with control was observed. Interestingly, 800 mg/kg EECC-treated rats and mifepristone-treated rats had 100% abortion and no foetus survival ratio (Table 3). This implies that 800 mg/kg EECC has a very potent abortifacient effect which is comparable with mifepristone.

Table 3. Abortifacient Activity of the Ethanol Extract of *Citrullus Colocynthis* administered to Pregnant Female Rats

Parameters	Control	MIFP	100$mgkg^{-1}$ EECC	200$mgkg^{-1}$ EECC	400$mgkg^{-1}$ EECC	800mg/kg EECC
Maternal initial weight(g)	162.96 ± 3.01^{a}	161.38 ± 2.57^{a}	165.32 ± 2.09^{ab}	162.88 ± 2.56^{a}	164.14 ± 3.20^{a}	167.24 ± 1.35^{b}
Maternal final weight (g)	195.16 ± 4.85^{a}	176.90 ± 1.65^{b}	195.72 ± 4.67^{a}	193.40 ± 4.49^{a}	194.26 ± 2.06^{a}	185.50 ± 2.59^{c}
Foetus Survival ratio (%)	100	0	81.48	66.66	18.75	0
No. of rats that aborted	0	5	1	2	4	5
Percentage aborted (%)	0	100	20	40	80	100

Mean (n=5) ± SEM. $^{b\text{-}c}$Test values are significantly different ($p<0.05$) from the control

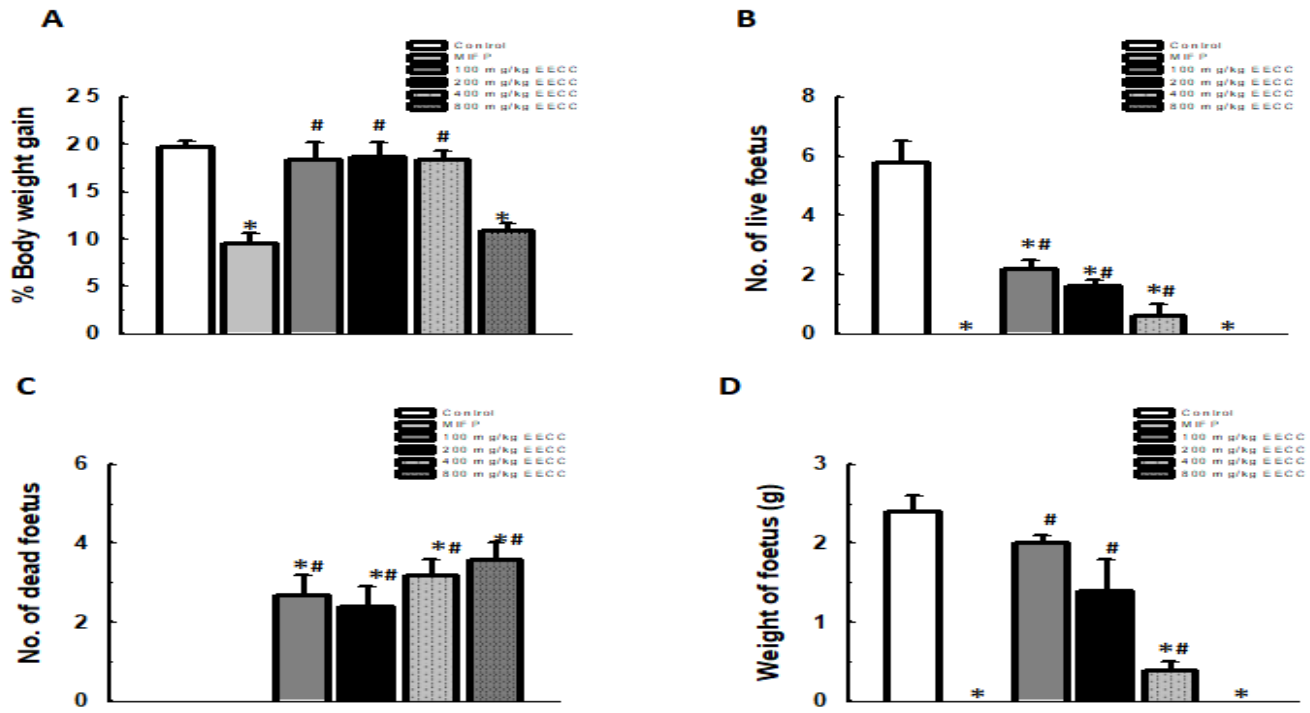

Figure 2(A-D). Shows the effect of EECC administration on (A) % Body weight gain (B) No. of live foetus (C) No. of dead foetus (D) Weight of foetus in pregnant Wistar rat. The results are expressed as the mean ± SEM, n = 5, $p < 0.05$; * and # indicates a value significantly different from control and MIFP group.

Effect of EECC treatment on foetal implantation and resorption

There was a drastic decrease in implantation sites and computed implantation index in the EECC-treated groups and mifepristone-treated group when compared with control, whereas 100 mg/kg EECC-treated rats had a similar number of implantation sites and computed implantation index when compared with control (Figure. 3A & B). There was increased foetal resorption across the EECC-treated groups and the mifepristone-treated group when compared with control, as depicted by the elevated number of resorption sites and resorption index (Figure. 3C & D). Furthermore, a decrease in *Corpora lutea* was observed in the 400 and 800 mg/kg EECC-treated pregnant rats (Figure. 4A) and the mifepristone-treated group when compared with the control. More so, the number of *Corpora lutea* in 100 and 200 mg/kg EECC-treated group was comparable with the control, but increased when compared with a mifepristone-treated group (Figure. 4A). Furthermore, the pre-implantation loss increased in the EECC-treated and the mifepristone group when compared with the control. However, the treatment containing 100 mg/kg EECC-treated rats had a comparable pre-implantation loss to the control. The treatment with 200-800 mg/kg EECC in the treated groups showed elevated pre-implantation loss compared to the mifepristone-treated group (Figure. 4B). Similarly, post-implantation loss increased in EECC-treated animals compared to the control. Mifepristone- and 800 mg/kg EECC-treated rats showed 100% post-implantation loss (Figure 4C).

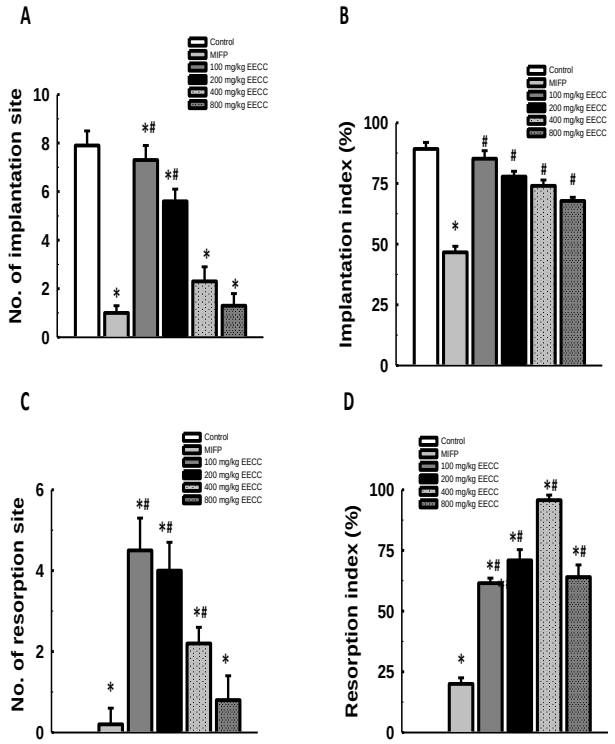

Figure.3 (A - D). The effect of EECC administration on (A) No. of implantation site (B) Implantation index (C) No. resorption site (D) Resorption index in pregnant Wistar rat. The results are expressed as the mean ± SEM, n = 5, $p < 0.05$; * and # indicates a value significantly different from control and MIFP group.

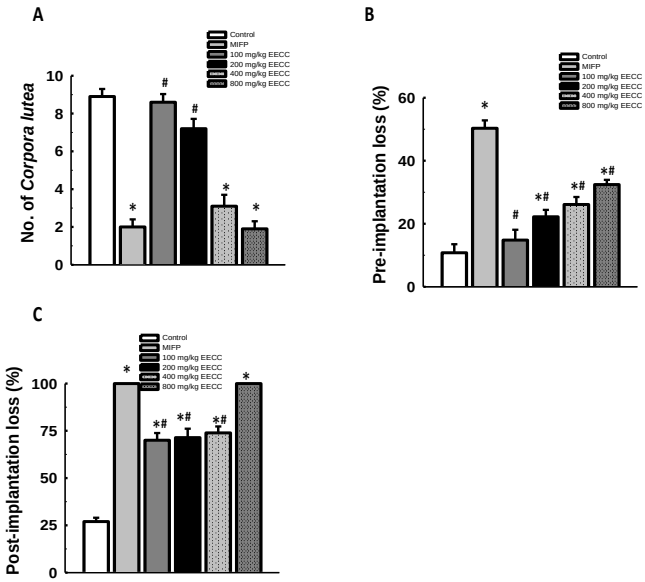

Figure. 4(A-C). The effect of EECC administration on (A) No. of corpora lutea (B) Pre-implantation loss (C) Post-implantation loss in pregnant Wistar rat. The results are expressed as the mean ± SEM, n = 5, $p < 0.05$; * and # indicates a value significantly different from control and MIFP group.

Effect of EECC treatment on reproductive hormones in pregnant rats

The levels of FSH, LH, prolactin, progesterone, and oestrogen decreased in a dose-dependent manner across the EECC and mifepristone-treated with the exception of the group treated with 100 mgkg^{-1} EECC in comparison with the control (Figure 5. (A-C) & 6 (A &B)).

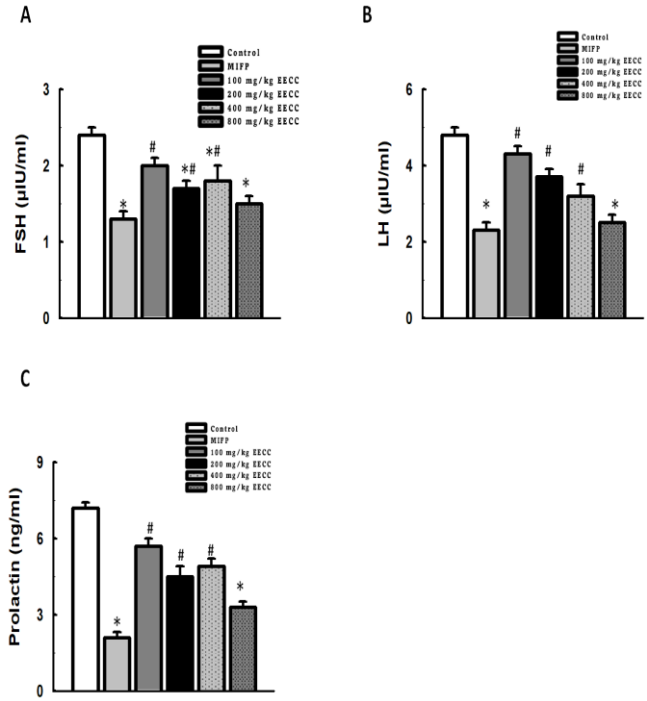

Figure. 5(A-C). The effect of EECC administration on (A) FSH (B) LH (C) Prolactin in pregnant Wistar rat. The results are expressed as the mean ± SEM, n = 5, $p < 0.05$; * and # indicates a value significantly different from control and MIFP group.

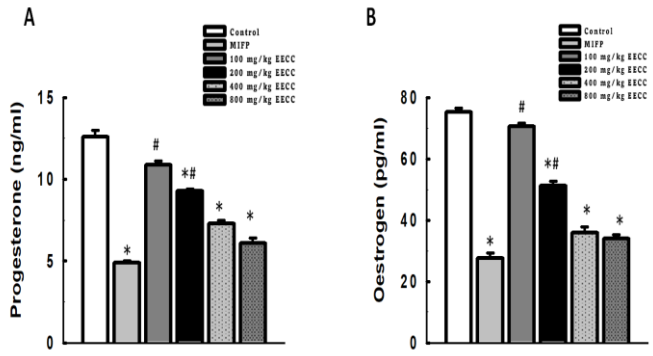

Figure.6(A-B). The effect of EECC administration on (A) Progesterone (B) Oestrogen reproductive hormone in pregnant Wistar rat. The results are expressed as the mean ± SEM, n = 5, $p < 0.05$; * and # indicates a value significantly different from control and MIFP group.

Effect of EECC treatment on the histology of the uterus of pregnant rats

The photomicrograph in Fig. 7A revealed that the uterus of rat treated with 0.5ml of olive oil showed that some layers and gland were not affected by the control treatment. Typical examples of these layer and organs includes functional is layer (white brace), basalis layer (black brace), and myometrium (green brace) with the presence of large and coiled uterine gland (yellow arrow), coiled arteries (red arrow) respectively while Figure. 7B showed that the three-layer of the uterus the endometrium (black brace), and myometrium (green brace), serosa (blue arrow) treated with 1.84mg/kg mifepristone showed the presence of large and coiled uterine gland (white arrow) and coiled arteries (blue arrow). In Figure. 7C the uterus of rat treated with 100mg/kg EECC had numerous blood vessels in the myometrium (red arrow), intact endometrium layer (black brace) with the presence of large and coiled uterine gland (yellow arrow). Moreover, Figure. 7D treated with 200 mg/kg EECC caused mild vacuolization of the myometrium (green arrow) with intact endometrium layer (blue brace) and presence of large and coiled uterine gland (white arrow). The photomicrograph in Figure. 7E and 8F shows that treatment with 400 and 800 mg/kg EECC to rats shows mild degeneration of the simple columnar epithelium lining the uterine gland (green arrow) and also the supporting connective tissue is loose and less dense (red arrow).

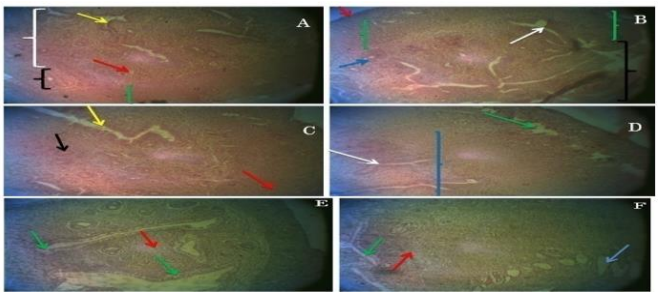

Figure 7. Microscopy of uterus tissue in different groups. Representative photographs of H&E staining in control group (A), Mifepristone group (B) 100 mg/kg EECC (C), 200 mg/kg EECC group, 400 mg/kg EECC group and 800 mg/kg EECC group in Wistar rat uterus (D) (400X; H &E staining).

DISCUSSION

In developing countries about eighty percent of the population depend on traditional medicine from plant origin to addresss basic medical needs that could not be accessed due to highcost synthetic drugs (Kaur et al., 2011). Notably, a number of bioactives derived from natural sources; like plant have been documented to have the potential to prevent or terminate pregnancy. The contragestive property of the ethanol extract of Citrullus colocynthis and its associated mechanism was therefore examined in this study. It was revealed that there are many phyto-constituents like alkaloids, saponins, phenolics, steroids, glycosides, flavonoids, tannins, and phlobatannins in EECC, which either work alone or together. This is corroborated by the reports that secondary metabolites like alkaloids, phenolics, saponins, steroids, and glycosides could work either singly or in combination to initiate pregnancy-terminating effects (Yakubu et al., 2010; Srikanth et al., 2013). Also, phenolics, phytosteroids, and saponins were confirmed to possess anti-fertility and abortifacient activities in rat models (Jain et al., 2013; Uboh et al., 2010). The EECC also contain cucurbitacins a member of tetracyclic triterpenoid substances with numerous pharmacological benefits. Hence, decrease in foetus survival ratio with increase in EECC dosage might support it potential to induce abortion in rats by disrupting pregnancy or altering foetus development. This is supported by the report of Liu et al. (2018) that cucurbitacins can caused cell cycle arrest. Also, cucurbitacine have been reported to have antifertility potential (Lavie and Glotter (1971).

In addition, there is a reduction in body weight gained by the pregnant rats administered with 800 mgkg-1 EECC and MIFP, this could be a signal to a loss in maternal homeostasis integrity and intrauterine growth restriction, which suggest that the foetus growth may be hindered. This agrees with the observation of Aprioku and Nwogu (2018) study to examine the *Piper guineense* abortifacient Potential. Also, the decrease observed in live foetuses with an increase in EECC dose could contributes to the maternal weight. However, no physical signs of toxicity were recorded except for the mifepristone-treated group, which showed signs of tiredness, which further supports the symptoms of the drug. It is therefore suggested that the EECC seems to be more tolerated by the animals than mifepristone. Also, the decrease in the live foetus, foetus weight, and foetus survival ratio could be linked with the abortifacient potential of EECC. However, the increased number of dead foetuses could be attributed to the observed increase in percentage post-implantation loss in the animals, thus demonstrating the contragestive activity of EECC. This agrees with Shaikh et al. (2015) report on *Anthocephalus cadamba* (Roxb.) Miq stem bark abortifacient potential in mice.

The implanted blastocytes within the uterus were estimated using the implantation index and pre-implantation loss, while the resorption index, as well as the post-implantation loss, reflects the connection that exists between the implanted and undeveloped blastocytes (Almeida and Lemonica, 2000). However, the decrease in the implantation sites with respect to the increase in EECC dosages is suggestive of the anti-implantation consequence of the extract and mifepristone, this is corroborated by the findings of Qazan et. Al. (2007). Interestingly, this led to a decrease in the percentage implantation index. Furthermore, a reduction in implantation index with corresponding elevation in the pre- and post-implantation losses is dose-dependent, supporting the foetal resorptive properties of EECC. There is an increase in the percentage resorption index with increase in dose of EECC. The observed increase in foetal resorption index shows that there was disruption of the implanted foetus in the pregnant rats and this is supported with the findings of Elbetieha (2000) and Shaikh et al. (2015). Also, Dhanwad et al. (2005) reported increase in percentage resorption index is a signal to failed embryo development. Hence, it could be due to uterine milieu modification that could lead to early resorption or late foetal mortality as documented by Kaur et al (2023). Moreover, the decrease observed in the corpora lutea in the EECC-treated groups, as well as mifepristone, implies that the mother's reproductive ability was altered, leading to the anti-pregnancy potentials of the extract and mifepristone. These results have shown that EECC exhibited pregnancy-terminating potentials.

The reproductive hormones like progesterone, oestrogen, FSH, LH, and prolactin were quantitatively analysed in the current study to diagnose pregnancy-terminating potentials of EECC. However, follicle-stimulating hormone serves as the prime reproductive hormone in all mammals. It is involved in the development of gonads in females, maturation at the time of puberty, and the genesis of mature Graafian follicles during the fertile period (Brain and John, 2007). The hormone acts on the granulosa receptors present on the cells directly. Meanwhile, the observed reduction in FSH might be connected to an impairment in the animal reproductive cycle as well as failing pregnancy. Therefore, the decrease in FSH caused by EECC and mifepristone could obstruct the synthesis of follicles and disrupt the maturation or development of the follicle at the pre-ovulatory phase (Yakubu et al., 2005). The reduction in FSH by the EECC could be one of the mechanisms underlying abortion and resorption in pregnant rats.

Luteinizing hormone (LH) triggers the gonads to release the sex hormones and maintains the corpora lutea. However, the activity LH triggers the production of androgens by the thecal cell, which are substrates for oestrogen synthesis by granulosa cells (Choi and Smitz, 2014). Meanwhile, LH secretion during the pre-ovulatory period suddenly surges and stimulates ovulation, causing the ovary's developed follicles to rupture. Therefore, the sudden decrease in serum LH levels may suggest that LH release was inhibited by EECC, suggesting ovulation disruption (Cole, 2009; Melmed et al., 2011; Goncailves et al., 2012). This can result in an impaired estrous cycle, which could hinder conception or result in abortion.

Progesterone also regulates the menstrual cycle and prepares the uterus for conception and pregnancy (Wang

and Dey, 2006). The hormone promotes the lactation glands during pregnancy while maintaining the uterine milieu for implantation, egg maturation, and the prevention of the subsequent release of the egg until termination of pregnancy (Spencer and Bazer, 2004). The inactivation or inhibition of the ovarian follicles' luteinisation caused by EECC in this study would have been responsible for the observed decline in progesterone. This is supported by Sharma et al. report that herbal contraceptive work by disrupting the uterus function or blocking reproductive hormone production (Sharma et al., 2015). Furthermore, the decline in progesterone in the EECC-treated groups and mifepristone is suggestive of impaired uterine endometrial support for foetal development.

Oestrogen stimulates the proliferation of the uterine lining and increases its thickness during the pre-ovulatory stage in the female cycle. The growth and development of reproductive organs are mostly achieved by oestrogen. Meanwhile, FSH in conjunction with oestrogen stimulates granulosa cell proliferation during follicular development. Therefore, a decline in oestrogen level could be attributed to reduced oestrogen synthesis, which implies that EECC could block ovulation by directly acting on the pituitary gland and influencing LH and FSH peripheral modulation, thereby decreasing the release of these hormones. Consequently, the decline in oestrogen observed can block or inhibit ovulation, zygote implantation, and continuous maintenance of pregnancy (Reed and Carr, 2000; Xu et al., 2017).

The hormone prolactin functions in the implantation, decidualization, and further placentation of the endometrium. Prolactin has also been reported to be one of the hormones upregulated during decidualization and the most predominant amniotic fluid secretory products (Bao et al., 2007; Gellersen et al., 2007). Therefore, the decrease in prolactin by EECC could support it contragestive potential. Meanwhile, Garzia and colleagues reported that the lack of endometrial prolactin during the implantation process seems to be implicated in reproductive failure.

Moreover, EECC was discovered to be responsible for the degeneration of the simple columnar epithelium lining and loss of the uterine supportive tissue of the uterine gland; this might be due to the alteration in the reproductive hormones or the bioactive constituents of EECC (Padmashali et al., 2006). Hence, the mechanism of action involved in the termination of pregnancy could be through the arrest of all follicular development responsible for the development of athretic follicles.

CONCLUSION

This present study demonstrated that the ethanol extract of *Citrullus colocynthis* possess contragestive activity in pregnant rats. It is noteworthy that 400 and 800 mg/kg EECC showed higher abortifacient activity compared with mifepristone. Consequently, it could be suggested that the phytoconstituents present in *Citrullus colocynthis* work singly or in combination to trigger pregnancy termination process. Interestingly, the abortion process is mediated through alterations in reproductive hormones associated with impaired uterine milieu, luteolysis, and delay or blockage of ovulation, respectively.

AUTHORS' CONTRIBUTIONS

JBA and EAB conceived and designed the experiments. JBA conducted the experiments and carry-out statistical and data analyses. JBA. JBA drafted the manuscript. JBA and EAB revised and edited the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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