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The uterotonic screening of calyx extracts of *Hibiscus sabdariffa* on estrogenized isolated Uterus of Wister strain albino rats

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Abstract

The claim of the use of orally consumed concentrated *Hibiscus sabdariffa* calyx preparation for the purpose of abortion in view of its widely uses as a beverage in the present period of economic hardship where it is a preferred beverage due to its affordability, naturalness and for its numerous medicinal properties has posed another element of concern to the female folks. This study was aimed to screen extracts of *H. sabdariffa* calyx of its acclaimed uterotonic activity. Contractile responses of estrogenized isolated rat uterus using various volumes (0.1, 0.2, 0.4, 0.6, 0.8, 1.0,1.6 ml) were established with oxytocin ($1x10^{-4}$ g/ml), ergometrine ($1x10^{-4}$ g/ml), misoprostol ($1x10^{-4}$ g/ml), Acetylcholine ($1x10^{-3}$ g/ml) and five different extracts of H. sabdariffa - ethanol ($2.0x10^{-3}$ g/ml), methanol ($2.0x10^{-3}$ g/ml), acetone ($2.0x10^{-3}$ g/ml), cold water ($2.0x10^{-3}$ g/ml) and hot water ($2.0x10^{-3}$ g/ml). After these, nifedipine, acetylcholine and salbutamol were used to challenge the maximal responses of the isolated uterine tissue to oxytocin, acetylcholine and the cold aqueous extract of H. sabdariffa (CWEHs). The CWEHs was equally potent at inducing contraction activity compared to the standard drugs. This study further shows reduction in the amplitude of contraction produced by CWEHs in the presence of Promethazine (1×10^{-6} g/ml) and Nifedipine (1×10^{-6} g/ml). The blockade is more pronounced in promethazine compared to Nifedipine. Aqueous extract of *Hibiscus sabdariffa* calyx possess uterotonic activity on estrogenized isolated rat uterine muscle with mechanism of action related to histamine receptors and contraction of the myometrial cells as the was antagonized by promethazine and Nifedipine. This could be a justification for its alarming use as an abortifacient.

Keywords: Abortifacient; Uterotonic; *Hibiscus sabdariffa*; Caution; Beverage; Cold water

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1 Introduction

The uterus is a hollow organ that provides protective and nutritive support to the ovum from the moment it is fertilized until it grows into a well-developed fetus ready for parturition. Its middle muscular layer is called the myometrium, which is known for its rhythmic contractions which result in 'endometrial waves' in the nonpregnant uterus, Braxton Hicks contractions during pregnancy, and true labor towards the end of the third trimester [1]. Abnormal uterine contractility may underlie important clinical pathologies such as infertility, implantation failure, ectopic pregnancies, spontaneous miscarriages, preterm birth, and caesarean section [2]. Miscarriage, also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently [3]. In other words, abortion refers to the spontaneous or deliberate ending of a pregnancy before the fetus has evolved sufficiently to survive. Overall, abortion is the termination of pregnancy before 20 weeks of pregnancy [4]. The causes of abortion include embryonic factors including chromosomal abnormalities and abnormal evolution of the zygote, and maternal factors including uterine anatomical abnormalities, immunological factors, coagulation disorders, endocrine factors, infections, environmental factors, physical trauma and cervical failure [5]. Induced abortion is the deliberate termination of pregnancy using medical or surgical procedures before the embryo can survive. In Nigeria, abortion is only legal to save a woman's life. Recent estimates extrapolated from facility-based abortion complications indicate there were approximately 33 abortions per 1000 women aged 15–49 in 2012 [6]. Findings from gynaecological admissions at nine referral hospitals in Nigeria suggest that, although surgical abortion is still the primary method of abortion, the share of postabortion care (PAC) patients who report first using misoprostol is increasing [7]. Furthermore, among gynaecological admissions at a Nigerian teaching hospital in recent years, 7.4% were related to treatment of unsafe abortion, 17% of which ultimately resulted in maternal death [8]. Efforts to increase awareness of the availability of medication abortion drugs to more safely self-induce can help mitigate the toll of unsafe abortion-related morbidity and mortality [9]. It has been shown that over 60% of unintended pregnancies among adolescents in secondary school will invariably end up in induced abortion [10]. Lamina *et al*, (2015) also reported the prevalence of unintended pregnancy as 35.9% while that of induced abortion was 33.5% also with the country's economic condition often cited as reasons women (15-48years) make use of abortion services for among women in South-Western Nigeria [11].

The foregoing are indicators that abortion in Nigeria is a public health concern as it affects both the young and the old both directly and indirectly, thus a social challenge. Herbal medicines are also emmenagogues (to avoid uterine flow) and oxytocic's (to stimulate uterine tightening, predominantly to induce labor) [12]. The use of herbal medicine has been on the increase in many developing countries [13]. These herbal remedies are used due to their cost effectiveness and ease of access [14]. The percentage of women depending on herbal medicine for their healthcare needs in developing countries is about 80% [15]; restricting its use by pregnant women has been a herculean task. Most pregnant women assume that nature-based medications do not lead to any drug interaction for fetal and maternal and therefore turn to self-medication with them, while they may cause certain fetal and maternal side effects or drug interactions [16]. Indigenous varieties of plants from various families are used by pregnant mothers in rural areas and low-income populations of sub-Saharan Africa for their uterotonic potential [17]. Despite the fact that majority of local herbal remedies used by sub-Saharan African pregnant women have never been botanically identified [18], several plants have been screened for their uterotonic activity using *in vitro* methods with positive results [19]. Report from a community visit showed that adolescent girls orally consume high concentration of *Hibiscus sabdariffa* calyces drink for abortion.

The genus *Hibiscus* (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees [20] including *Hibiscus sabdariffa (Hs)* [21], which became domesticated and widely cultivated West Africa, cutting across both tropical and subtropical regions especially Sudan, Nigeria, Egypt as well as Mexico in the Americas [22, 23]. *Hs* is easy to grow in most well drained soils but can tolerate poor soils. In Sudan and Nigeria, the calyces are boiled with sugar to produce a drink known as "Karkade" or "Zoborodo" [24]. Both clinical and non- clinical studies revealed some medicinal properties of *H. sabdariffa*, including decreased systolic and diastolic blood pressures in those patients [25], anti-inflammatory activity [26] antioxidant [27], anti-diabetic [28], antinociceptive effect in a rat model [29], anti-microbial [30] e.tc

The claim of the use of orally consumed concentrated *Hibiscus sabdariffa* for the purpose of abortion in view of its widely used as a beverage (Zoborodo) in the present period of economic hardship where it is a preferred beverage due to its affordability, naturalness and for its numerous medicinal properties has posed another element of concern to the female folks thus urgent need for pharmacological screening for uterotonic activity as the aim of this study.

2 Material and methods

2.1 Study Site

This study was carried in the Pharmacology Laboratory, Department of Pharmacology and Therapeutics, College of Medicine and Allied Health Sciences, Bingham University, Jos, Nigeria.

2.2 Plant material procurement and preparation

Dried leaves of *Hibiscus sabdariffa* was purchased from Terminus Market, Jos North LGA, Plateau State, Nigeria.

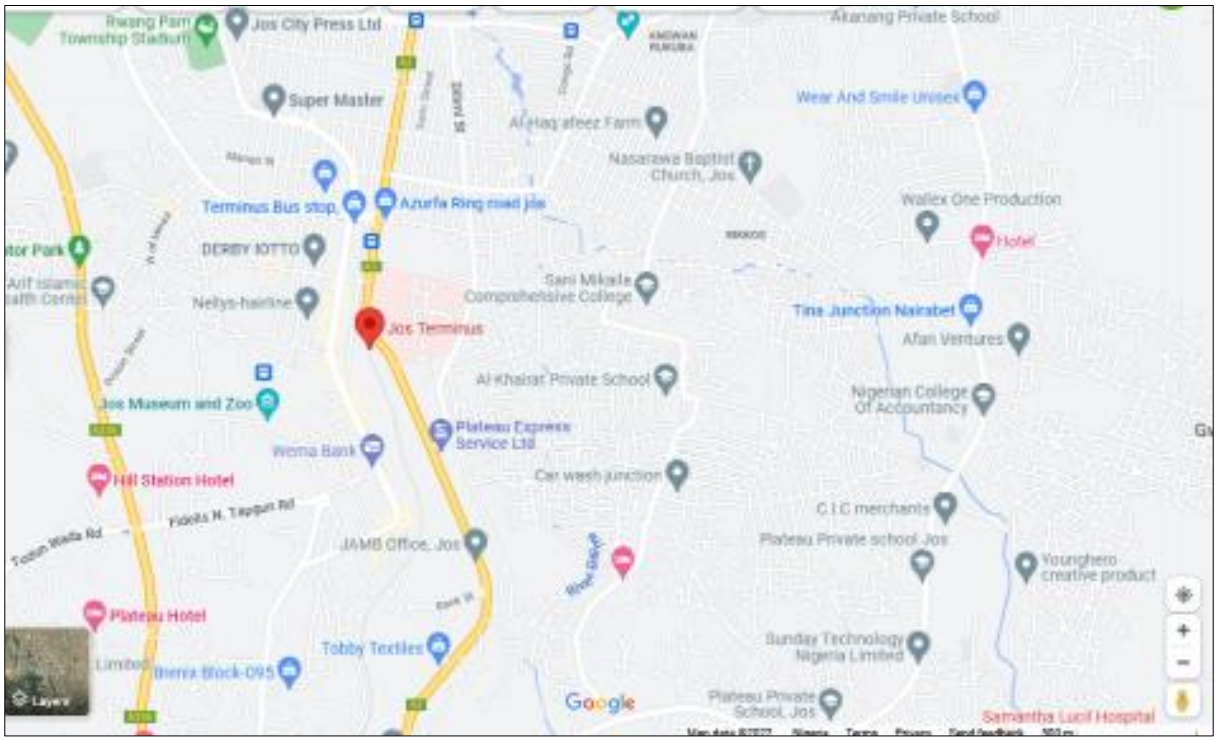

Figure 1 Google map of Terminus Market [31]

Figure 2 Dried calyx of *H. sabdariffa* [32]

Figure 3 Fresh plant of *H. sabdariffa* [33]

2.3 Plant material procurement and Preparation of *H. sabdariffa* Leaves Material

The procured dried calyces of *H. sabdariffa* were gently rinsed with clean water and dried under ambient light and temperature in a well-ventilated environment for 5 days, after which the dried leaves were ground into powder using mortar and pestle, The powder was then sieved into fine powder using size 80 Sieve. The Powder of plant material was stored in dark bottle container until required for use.

2.4 Extraction of *H. sabdariffa* Leaves Material

Five different extracts using different solvents (ethanol, methanol, acetone, cold water and hot water) were obtained. Powder sample of *H. sabdariffa* (100g) each was weighed and soaked in five different jars containing 700ml each of ethanol, methanol, acetone, cold water and hot water respectively stirred by way of extraction (maceration) for 72 hours. The extracts were filtered using Whitman No.1 filter paper while the crude extracts were then evaporated to dryness using a rotary evaporator and percentage yield calculated to obtain a powder whose yield was calculated. The five extracts eventually parked into dark bottles and stored in a refrigerator at 4°C according to the method of Idyu *et al.,* 2015 [34] and Builders *et., al* 2016 [35]. Until required for use.

$$\text{Percentage yield} = \frac{\text{Mass of pure powder}}{\text{Mass of impure}} \text{X } 100$$

From the extraction of the extracts of *H. sabdariffa*, the yield was cold water (15.3%), Hot water (12.93%), ethanol (11.60%), methanol (8.20%) and acetone (4.1%) respectively.

2.5 Acute Toxicity Test

The LD_{50} of the ethanol, methanol, acetone, cold water and hot water *H. sabdariffa* extracts were determined following Lorke's model [36]. Results from the phase 1 and 2 shows $10 \leq x \leq 5000$ for all the extracts.

2.6 Experimental Animals

Healthy non pregnant adult female rats (Wistar strain) rats between 10 and 12 weeks old and weighing 150–170 g were obtained from the Animal House Unit of the Department of Pharmacology and Therapeutics, College of Medicine and Allied Health Sciences, Bingham University, Jos, Nigeria. The animals were housed in plastic cages, allowed free access to pelleted animal feed and clean water *ad libitum* for 7 days for acclimatization in the Pharmacology Laboratory. This study was out carried in the Pharmacology Laboratory of the College of Medicine and Allied Health Sciences, Bingham University, Jos, Nigeria.

2.7 Standard Drugs and Physiological Salt solution Used

The following drugs were used for this study:

- Diethylstilbestrol injection (Kunj Pharma pvt, Ltd., India) was used to estrogenize the nongravid Wistar rats.

- Oxytocin injection (Rotex Medica), Ergometrine (Hameln. UK.) And Misoprostol (Marie Stop Int) were used as a standard uterotonic drug.
- Acetylcholine (Sigma Aldrich Germany).
- Atropine injection (Sigma Aldrich Germany) was used to block the muscarinic receptors of the uterine smooth muscles.
- Nifedipine (Krishat Pharma Ind. Nigeria) – Inhibitor of Calcium channel blocker
- Indomethacin (Greenfield Pharm Ltd. China)
- Promethazine (JinLing Pharmaceutical, China)
- Freshly prepared De Jalon solution of the following composition: NaCl (9.00 g), KCl (0.42 g),
- CaCl2 (0.06 g), NaHCO3 (0.50 g) and glucose (0.50 g).

2.8 Animal Preparation/ Isolated Organ Preparation

The animals were estrogenized with 0.2 mg/kg diethylstilbestrol (DES) intraperitoneally (i.p). The drug was reconstituted with ethanol/water (1:1) solution prior to drug administration. Twenty-four hours later, the rats were sacrificed under chloroform anesthesia, had the uterine horns isolated devoid of excess fat and connective tissues, and cut into longitudinal strips. Then the uterine muscle strips were suspended with one end attached to a tissue holder in a 50 ml capacity tissue bath containing De Jalon's physiological solution. The other end of the strips was connected to an isotonic transducer and in turn connected to a 3 channel microdynanometer Power Lab (BD Instruments – ISO 9001:2000). The entire organ bath was maintained at 37°C and aerated with a mixture of 95% oxygen (O_2) in 5% carbon dioxide (CO_2). Having allowed the preparation to equilibrate for 30 minutes, tissue activity was monitored and observed via the microdynanometer recording paper before and after an intervention.

Contractile responses using various volumes (0.1, 0.2, 0.4, 0.6, 0.8, 1.0,1.6 ml) were obtained with oxytocin (1×10^{-4} g/ml), ergometrine (1×10^{-4} g/ml), misoprostol (1×10^{-4} g/ml), Acetylcholine ((1×10^{-3} g/ml) and five different extracts of *H. sabdariffa* - ethanol (2.0×10^{-3} g/ml), methanol (2.0×10^{-3} g/ml), acetone (2.0×10^{-3} g/ml), cold water (2.0×10^{-3} g/ml) and hot water (2.0×10^{-3} g/ml). After these, nifedipine, acetylcholine and salbutamol were used to challenge the maximal responses of the isolated uterine tissue to oxytocin, acetylcholine and the cold aqueous extract of *H. sabdariffa*.

The mechanism of *H. sabdariffa*-induced uterine contractions by way of cholinergic, histaminergic and oxytocinergic pathways were ascertained in the presence of antagonists by pre-treatment with atropine (1×10^{-6} g/ml), Promethazine (1×10^{-6} g/ml) used to block H_1 receptors, Nifedipine (1×10^{-6} g/ml) used to block the L-type calcium channel and Indomethacin (1×10^{-6} g/ml) used to inhibit cyclooxygenase (COX) enzymes of the uterine smooth muscles.

3 Result and Discussion

The present study shows that the hot water, ethanolic, methanolic and acetone extracts of *Hibiscus sabdariffa* most probably denatured the active ingredients in the plant sample, this suggests the reason for low percentage yield and zero contraction of the uterine muscle, in other words, administration of increasing doses of hot water (2×10^{-3} g/ml), ethanol (2×10^{-3} g/ml), methanol (2×10^{-3} g/ml), and acetone (2×10^{-3} g/ml) produced no contraction on the uterine smooth muscle and may be due to the fact that the active constituents in *Hibiscus sabdariffa* are more soluble in cold water. However, the cold-water extract has higher percentage yield (15.3%) and uterotonic activity at concentration of 2×10^{-3} g/ml on the isolated tissue.

Uterine smooth muscle often undergoes spontaneous rhythmic contractions that change in amplitude and frequency. Addition of increasing volumes of Misoprostol (1×10^{-4} g/ml), Oxytocin (1×10^{-4} g/ml), ergometrine (1×10^{-4} g/ml) dose dependently enhanced the contraction activity of the uterine muscles (amplitude and frequency), and produced a graded dose response tracing. Similarly, the CWEHs (2×10^{-3} g/ml) produced a dose dependent contraction of the rat uterine smooth muscle. The volume that gave the maximal response was 0.2ml (2×10^{-3} g/ml) – *Figure 4*. Administering greater than 0.2ml (2×10^{-3} g/ml) of the CWEHs is not likely to produce a more forceful contraction. One reason for this may be that the contractile mechanism is already running in a saturated manner. The responses of CWEHs were significant compared to the normal uterine contraction. The results of the standard drugs compared with the CWEHs was equally potent at inducing amplitude of contraction but the standard drugs were more potent especially oxytocin. The concentration-response curve gave sigmoidal with EC_{50} for CWEHs (3.2×10^{-5} g/ml), Oxytocin (1.3×10^{-4} g/ml), Ergometrine (4×10^{-5} g/ml), Misoprostol (1.6×10^{-4} g/ml), and Acetylcholine (3.2×10^{-6} g/ml). This study further shows reduction in the amplitude of contraction produced by CWEHs in the presence of Promethazine (1×10^{-6} g/ml) and Nifedipine (1×10^{-6} g/ml) – *Figure 5*. The blockade is more pronounced in promethazine compared to Nifedipine.

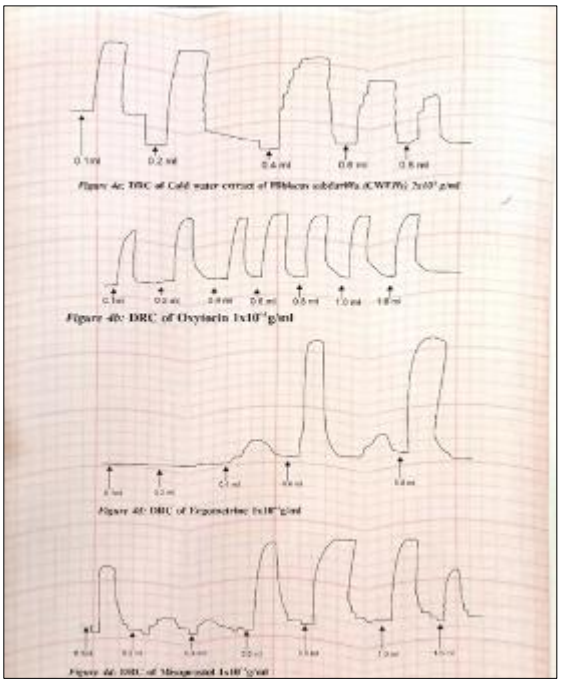

Figure 4 Dose- response curve (DRC) of CWEHs and standard drugs

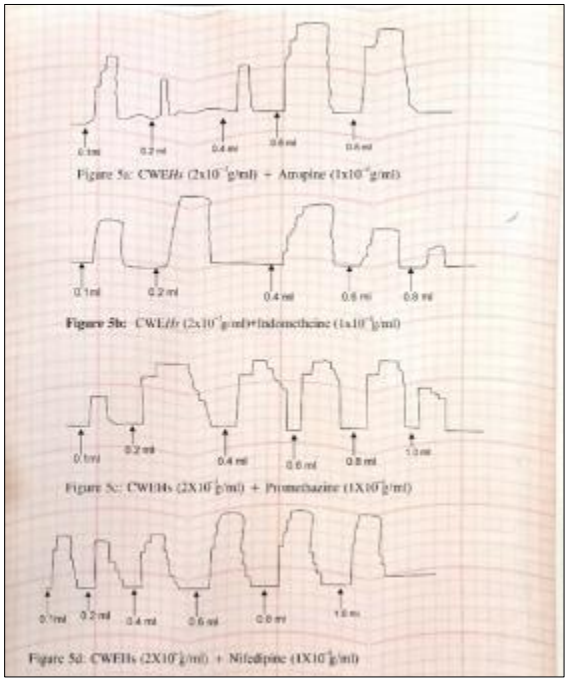

Figure 5 DRC of CWEHs in the presence of antagonists

Uterine contractile activity is regulated by the increase in intracellular Ca^{2+} concentration in the myometrial cells. Voltage-gated Ca^{2+} channels (VGCCs) mediate the Ca^{2+} influx in response to membrane depolarization and regulate intracellular processes such as contraction [37, 38] ,39]. Also, Histamine receptors (H_1 and H_2) are present in the uterus and the predominant response of histamine in this tissue is contraction (H_1 activity) [40]. The antagonism activity of CWEHs in the presence of Promethazine and Nifedipine suggests the likely mechanism of action to be via H_1 receptors and by calcium mobilization in the myometrium. Uterotonic activity of the cold water or cold aqueous extract of *Hibiscus sabdariffa*, even though at low volume as highlighted in this study is in consonance with the report by Fofie and Baffoe,

2010, on the prevalence of uterine rupture experienced by pregnant women as a result of the use of herbal preparations for inducing labour [41] thus, a concern for the pharmacology, public health, gynaecology, and other field of medicine community. Mode of preparation before consumption of the plant product should be looked into, women in their third trimester should apply caution in taking beverages especially such prepared via soaking *H. sabdariffa* cold water. While the results presented convincingly show that the cold aqueous extract of *H. sabdariffa* induces contractions in uterine smooth muscle tissue in vitro, further information is warranted in determining whether if the same results can be seen *in vivo* [42] when the herbals are ingested as water, spirit, or oil-based medicines [43].

4 Conclusion

Aqueous extract of *Hibiscus sabdariffa* calyx possess uterotonic activity on estrogenized isolated rat uterine muscle with mechanism of action related to histamine receptors and contraction of the myometrial cells as the was antagonized by promethazine and Nifedipine. This could be a justification for the alarming use as an abortifacient.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All the authors of hereby declare no conflicts of interest regarding this study.

Statement of ethical approval

Ogundeko T.O, Okoye NP among the authors and Kamoh L are licensed to handle laboratory animals thus, standard protocols involving the use of laboratory animals were strictly adhered to.

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