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Green Tea Inhibits Uterine Contractility in *Ex Vivo* (Non-Pregnant) Mice Models

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ABSTRACT

Green tea is widely known for its beneficial biologic effect and there have been some reports on the beneficial effect on the reproductive system. There have however been no reports on green tea effects on uterine contractility. This study is therefore aimed at the investigation of green tea extract on the amplitude and frequency of uterine contractility.
Green tea bags were macerated in boiling water for 5 min and concentrated to dryness. The extract (0.33 -1333.21 µg/mL) was tested on the isolated mouse uterus. The contractility parameters investigated included spontaneous contraction, oxytocin (60 pg/mL) induced contraction and high KCl (80 mM)-induced contraction. High resolution mass spectrometric (HRMS) determination of secondary metabolites was also performed on the extract.
The extract inhibited both the amplitude and frequency of uterine contractility studied, however minimal inhibitory effect was observed with KCl-induced contraction. The HRMS analysis revealed the presence of twenty-five (25) significant compounds, 23 of which were identified and 2 were unknown. Compounds were observed to belong to a diverse range of phytochemical classes including pteridine, flavonoids, cyclitols, and coumarins, with the majority of detected compounds belonging to the flavonoid class.
The results obtained in this study have shown that green tea extract inhibits uterine contractility possibly due to the presence of the flavonoids, and through interaction with calcium and/or prostaglandins.

Keywords: Green tea, Uterus, Oxytocin, Potassium chloride.

Introduction

Green tea is obtained from the tea plant *Camellia sinensis* Var. *sinensis* of the family Theaceae and is cultivated in several countries around the world.[1,2] Tea has been reported to contain over 4000 active metabolites and a third of this number are polyphenols.[3] Of the polyphenols, the flavonoid family of compounds constitutes the basic phenolic compounds in green tea which also contribute to the antioxidant activity exhibited by green tea.[4] Green tea has been reported to exhibit cancer prevention,[5] antioxidant activity,[6] antimutagenic,[7] and enzyme inhibition[8,9] activities. Beneficial effects of green tea on the reproductive system are also emerging. Traditional cultures worldwide depend on herbal remedies for different conditions in pregnancy, birth and post-partum care.[10] This therefore makes documentation of traditional knowledge of herbal remedies and scientific investigation relevant as it assists chemists and pharmacologists with starting points for "targeted" analysis, discovery of novel therapies and natural drugs for the treatment of pregnancy and related issues.[11] Green tea extract has been reported to significantly improve sperm motility, concentration and sperm membrane integrity after 28 days of green tea extract administration.[12] Though there have also been some contrary reports. For instance, Chandra and colleagues reported a significant decrease in epididymal sperm number, serum testosterone level in a dose-dependent manner and testicular steroidogenic enzyme activities in green tea extract -administered group of animals after 26 days.[13] Green tea extract has also been reported to improve oestradiol valerate-induced polycystic ovarian syndrome in rat, which is a major cause of infertility. In one study, green tea was observed to regulate gonadotropin levels, reduce insulin resistance and body weights as well as improve the ovarian morphology.[14,15] Similarly, a study reported the improvement of leiomyoma prognosis.[16] There has however been no study showing the effect of green tea on uterine contractility despite reports on male reproductive parameters. The uterus provides the protective environment for the developing fetus and then at term with the onset of labour, it contracts rhythmically to expel the fetus and placenta.[17] This, therefore, supports the study into uterine contractility as a female reproductive parameter that can be targeted for therapeutic interventions that can increase the quality of life for females and reduce complications of labour in the pregnant female.
In the search for potential targets and therapies in managing dysfunctions of uterine contractility, this study was therefore designed to investigate the activity of green tea extract on uterine contractility and to also investigate possible mechanisms of action. This study is also aimed at identifying significant secondary metabolites present in green tea that may have contributed to the biological activities observed.

Materials and Methods

Green tea extraction
Green tea bags (375 g) of the Lipton brand® (Unilever, USA) were obtained from a local supermarket in Benin City, Nigeria. The threads

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of the bags were trimmed off and the bags were macerated in distilled water (6 L) and heated at 100ºC for 5 min in order to mimic the method used for tea steeping prior to consumption. After 5 min heating, the mixture was allowed to cool and was decanted and sieved. The fluid tea extract was then concentrated by placing on a water bath set at 80 ºC. The concentrate obtained was dried in an oven set at 40°C and the extract weight was determined to be 122.3 g (percentage yield was 32.62% w/v). The extract was then stored in the refrigerator (≈ 4°C) till needed.

Animals

Mature non-pregnant female albino mice weighing between 20-30 g were obtained from the Animal House Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. They were housed in plastic cages at an environmentally controlled room temperature of approximately 27 ± 5°C and lighting conditions. Ethical consent was obtained prior to the start of the experiments from the Faculty of Pharmacy Ethics Committee, University of Benin, Nigeria (EC/FP/016/04). The animals were handled as much as possible according to standards of the Public Health Service policy on humane care and use of Laboratory Animals.[18,19] The animals were maintained on a standard diet of animal pellets and clean tap water.

Contractility studies

Tissue preparation

Each mouse was administered 1.0 mg/kg diethylstilbestrol (DES) orally constituted in Tween 80 and distilled water (1:1), 24 h prior to the day of experiments. Dose and route of DES administration had been previously determined in our laboratory to effectively induce estrous[20]. On the day of the experiment, vaginal smears were obtained with the aid of a Pasteur pipette (0.1 mm), fixed with ethanol and stained with a drop of Gentian violet. The smears were then observed under a microscope using a X10 objective lens in order to ascertain the oestrous stage Animals in proestrus and oestrous stages were selected and humanely killed by cervical dislocation and the uterine horns were immediately excised and immediately placed into a petri dish containing previously warmed and aerated De Jalon's physiological salt solution (composition in M: NaCl 154.00, $NaHCO_3$ 5.95, D-glucose 2.78, KCl 5.63, and $CaCl_2 \cdot 2H_2O$ 2.05). The uterine tissues were cleaned of connective tissues and one horn dissected in half to obtain a segment of the uterine horn. Tissue lengths of approximately 1-2 mm each was obtained. The uterine segment was then mounted in a warmed 10 mL organ bath maintained at 37ºC and containing an aerated physiological salt solution.

Experimental protocol

The uterine tissues were mounted in organ baths and equilibrated under resting tensions of 4.90 mN for 30-45 min or till regular contractions were obtained. The force and frequency of uterine contractions in the longitudinal muscle layers were measured using a 7003E-isometric force transducer (Ugo Basile, Varese, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy).

Experiment on the effect of extract on spontaneous uterine contraction

The direct effect of cumulative concentrations of the extracts on uterine smooth muscle contractility was investigated. Concentration-response relationships were obtained using concentrations between (0.33 - 1333.21 µg/mL). A contact time of 3 min was allowed following each concentration of extract administered. After each set of administration, the tissues were washed 3 times and a wash-out period of 10 min was allowed before the next administration.

Experiment on the effect of extract on oxytocin-induced uterine contraction

The effect of the extract on oxytocin-induced uterine contraction was investigated.[20] Cumulative concentrations of the extract were tested in the presence of 60.0 pg/mL oxytocin. A contact time of 5 min was allowed after oxytocin administration before cumulative concentrations of the extract were added and a contact time of 3 min was allowed following each administration.

Experiment on the effect on high potassium chloride-induced uterine contractility

The effect of the extract was determined in the presence of high potassium chloride (KCl) (80 mM).[20] KCl (80 mM) was added to the bath containing the uterine tissues and left in contact for 5 min and without washing, the effects of cumulative concentrations of the extract (0.014 – 16.32 µg/mL) were determined.

LC-HRFTMS identification of constituents in extract

Liquid chromatography-high resolution Fourier Transform mass spectrometry (LC-HRFTMS) analysis was performed on a Dionex UltiMate-3000 (DIONEX, Sunnyvale, CA, USA) coupled to a ThermoScientific Exactive Orbitrap system (Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany). An ACE column C18 75 × 3.0 mm column from Hichrom Ltd., Reading, UK was used. Parameters used were as previously described [21]. Briefly, elution flow rate was set at 300 µL/min with water (A) and acetonitrile (B), both of which contained 0.1% formic acid. A gradient flow starting with 10% B and increasing to 100% B, in 30 min was used. The mobile phase was maintained for 5 min at 100% B, followed by equilibration of the column with 10% B. The resulting data files were sliced into positive and negative datasets using ProteoWizard [22] prior to data mining which was done with the use of MZmine 2.10 [23]. Peak detection was accomplished using the centroid mass detector and a noise level of 1000 was set. The chromatogram builder generated peak lists from the mass lists obtained from the previous step. The minimum time span was 0.2 min, minimum height was 10,000, and the *m/z* tolerance was set to 0.0001 *m/z* or 5 ppm. Chromatogram deconvolution was accomplished using the local minimum search algorithm with the following parameters: threshold (90%), search minimum in RT range (0.4 min), minimum relative height (5%), minimum absolute height (10,000), minimum ratio of peak top/edge (2), and peak duration range (0.2-5.0 min). The peak lists were de-isotoped using the isotopic peaks grouper with an *m/z* tolerance of 0.001 *m/z* or 5 ppm, retention time tolerance of 0.1 minutes (absolute), and a maximum charge of 2. The representative isotope was the most intense. The peak lists were then merged using the Alignment function. The weight for *m/z* and for retention time (RT) was 20, and the RT tolerance was 5%. The aligned peak lists were gap-filled using the Peak Finder, with an intensity tolerance of 1% and RT tolerance of 0.5 min (absolute) [24]. Adducts were identified, together with other complexes that may have formed. The chemical formulae of the peaks were predicted using the formula prediction tool developed by MZmine. ChemBioFinder version 13 (PerkinElmer Informatics, Cambridge, UK) was used to access hits from the database.

Data analysis

The mean frequency and amplitude were computed from contractions occurring at the last 3 min of the phasic contractions using the GraphPad Prism, (version 7.03; GraphPad software Inc, San Diego, CA, USA). Results were obtained as percentages of control applications (control=100%) where necessary and changes in force (amplitude) or frequency were expressed with respect to control (100%). All data shown were expressed as mean ± standard error of mean (SEM) and 'n' represents the number of animals. Significance was evaluated using appropriate t-tests, and where necessary, one-way analysis of variance with Dunnett's post hoc and P values ≤ 0.05 was taken to represent minimum significance in all cases.

In datasets with sufficient data points, mean log concentration-response curves were analyzed by fitting data to a variable slope logistic equation, using the following equation values Y = Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)). Where Y = response which starts at the bottom and goes to the Top in sigmoid shape, X= logarithm of concentration and IC_{50} is the concentration that gives a response half way between Bottom and Top.

Results and Discussion

Effect of green tea extract on spontaneous uterine contraction

The green tea extract, at concentrations used in this study, was observed to have an inhibitory effect on spontaneous uterine contraction at high concentrations (Figure 1A). An increase in tension of the uterus was observed at 13.22 µg/mL which was followed by decreases in contractility with subsequent increased concentrations (Figure 1A). The

inhibitory effect was not pronounced on the amplitude (Figure 1B) but rather on the frequency of contractions (B). The EC_{50} of green tea was calculated for the frequency as 88.88 ± 0.35 ng/mL.

Effect of green tea extract on oxytocin-induced uterine contractions

The effect of green tea extract in the presence of an agonist, oxytocin (OT) was investigated (Figure 2A). Green tea had minimal effect on the amplitude though a slight inhibition was observed at higher concentrations of 133.21 and 1333.22 µg/mL (Figure 2B). However, a significant decrease ($P < 0.001$) in the frequency of OT-induced uterine contractions in the presence of green tea was observed which was also more pronounced at higher concentrations of 133.21 and 1333.22 µg/mL (Figure 2C).

Effect of green tea extract on high KCl-induced uterine contractions

The effect of green tea extract in the presence of high KCl-induced uterine contraction was investigated (Figure. 3A). Green tea inhibited KCl-induced contraction though non-significantly (Figure 3B). Inhibitions were observed at concentrations of 13.22, 133.21 and 1333.22 µg/mL (Figure 2B).

Identified secondary metabolites in Green tea

The LC-HRFTMS results and database search (using Dictionary of Natural Products) enabled the detection of 25 significant compounds (Tables 1 and 2), 23 of which were identified (Table 1) while two compounds were unknowns (Table 2). The identified compounds were observed to belong to a diverse range of phytochemical classes including pteridine, flavonoids, cyclitols, and coumarins, with the majority of detected compounds belonging to the flavonoid class (Table 1) and ionizing in the negative mode (Figure 4). The peak at 14.52 detected in the positive mode was identified as a plasticizer, which was considered to be an artefact.

Green tea extract produced inhibitions of uterine contractility on the contractility parameters investigated in this study which included the amplitude and frequency of uterine contractions.

The smooth muscle layer of the uterus is constantly contracting all through the life of females and not limited to labour and/or delivery times. Contractility of the uterus takes place during the menstrual cycle in non-pregnant uterus (which may lead to dysmenorrhoea) and also during pregnancy particularly at term all of which occurs via a complex yet dynamic physiological phenomenon[25] supporting the study on non-pregnant uterus in this study. Dysfunction of the uterine smooth muscle often affects contractions leading to abortions or preterm delivery, in some cases these contractions are quite strong and lead to foetal distress, hypoxia and often death.[25,26] For the non-pregnant uterus (which was utilized in this study), contractions occur at different phases of the menstrual cycle; and are often referred to as spontaneous contractions. Focal and sporadic bulging of the myometrium,[27,28] which causes sustained contractions as observed with high KCl in this study also occurs. These uterine contractions are necessary for endometrial sloughing [29] and also supports passage of sperm [30]. Similar mechanisms of uterine contractility occur in late-term pregnancy with variations in the degree, as it does in the non-pregnant uterus, to systematically ensure successful expulsion of the foetus.[30] This then implies that regardless of the presence or absence of pregnancy, uterine contractions are dependent on the contractile activity of the cellular elements, the uterine myocytes. The uterine myocytes are uterine smooth muscle cells which exhibit phasic pattern of contractility in a manner to maintain the resting tone. The resting tone is usually superimposed by intermittent sets of contractions with fluctuating frequency, amplitude and duration. Intracellular calcium concentration ($[Ca^{2+}]i$) largely regulates this contractility [25,26] also referred to as spontaneous contractions. Green tea was observed in this study to inhibit these spontaneous uterine contractions at high concentrations which suggests an inhibition of the force and frequency of myocyte activity through $[Ca^{2+}]i$ inhibition. An increase in $[Ca^{2+}]i$ activates calcium ion (Ca^{2+})-dependent cytosolic protein, calmodulin (CalM).[31] A Ca^{2+}-CalM complex is formed which activates an enzyme, myosin light chain kinase (MLCK) causing an increase in myosin regulatory light chain-20 (MLC20) phosphorylation of and subsequent cross-bridge cycling.[32] Phosphorylation of MLC20 by MLCK is the principal determinant of the amplitude and duration of uterine contraction.[33,34] The inhibitory effect of green tea was more evident with the frequency than the amplitude which may be attributed to a minimal effect of green tea on MLC20 phosphorylation. MLCK activation by CalM is considered a rate-limiting step of contraction [35] and contributes to the contraction frequency of uterine smooth muscles. It would, therefore, appear that green tea may exert greater activity on MLCK activation and possibly a lesser effect on prevention of MLC20 phosphorylation which affects the electrical activity within the uterus and may explain the differences in effect of green tea on the frequency and amplitude of uterine contraction.

Green tea was also examined on OT-induced uterine contraction. Oxytocin is largely known to contract the uterine smooth muscle.[30] Calcium release and entry from the sarcoplasmic reticulum (SR) is augmented by OT. This is achieved by coupling of OT to its receptor which activates phospholipase-Cβ, leading to the hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to release inositol triphosphate (IP_3) and diacylglycerol (DAG).[36] Intracellular calcium from the SR is activated by IP_3 causing more opening of extracellular calcium channels. DAG proceeds to activate protein kinase C[36] all culminating in powerful uterine contractions. The observed effect of green tea on oxytocin in this study supports the developing hypothesis that green tea may be involved in the prevention of MLCK activation. Interaction of green tea with IP_3 and DAG is also hypothesized.

KCl-rich solutions depolarize the cellular membrane of muscles resulting in contraction.[37] Potassium–rich solutions alter the distribution of ions on myocyte membrane, activating an action potential which results in membrane depolarization and a markedly increased Ca^{2+} entry, with eventual contraction.[38] These activities lead to a 'complex action potential' consisting of an initial spike-like depolarization followed by a sustained depolarization plateau and also involves strong conduction of Ca^{2+} [38] through voltage-operated calcium channels (VOCs), particularly the L-type calcium channel.[39] Propagation of the action potentials through the uterine smooth muscle is coordinated by gap junctions within the myocytes prompting contraction synchronicity at the whole organ level.[38,40] Several calcium channel blockers inhibits this mechanical response to high K^+ via blockade of the L-type channels.[41] This therefore suggests a mild interaction of green tea with VOCs which would have been responsible for the mild inhibition of KCl-induced contractions by green tea observed in this study.

Several compounds were identified in green tea belonging to general classes of pteridines, cyclitols, flavonoids and coumarins. Some reported biological effects of some of the identified compounds are briefly discussed here in order to extrapolate possible relationship with the activity of green tea in this study. Pteridinedione belongs to the family of pteridines xanthopterin and are products of tetrahydrobiopterin degradation, a coenzyme utilized by aryl amino acid hydroxylases, glyceryl ether monooxidase and nitric oxide (NO) synthases.[42] Pteridines have been reported to exhibit cytotoxic effects on a number of cancer cell lines.[42,43] Pteridines have also been reported to inhibit NO production.[43] The reported inhibition of NO production may contribute to the increased tension observed at lower concentrations of green tea in this study. In the presence of heat, HMF can be produced from sugars in plants which can then be enzymatically broken down to produce FCDA.[44] This is a probable explanation for the detection of FCDA in green tea in this study. The biologic effect of FCDA is yet unknown and further studies are required to determine the effect of FCDA on uterine contractility. Hexahydroxyflavan and epicatechin are tannin flavonoids [45]commonly found in tea and are members of the class of compounds known as catechins.[46] Catechins are monomers of flavan-3-ol.[47] Specifically, 3,3',4,4',5,7-Hexahydroxyflavan is an epigallocatechin (ECG).[47] Flavonoids are considered the main biologically active constituents of green tea[47]. Flavonoids are reported to inhibit activity of prostaglandins and NO[48,49]. Some flavonoids have been reported to inhibit lipoxygenase[17] and NO synthase[50] therefore preventing smooth muscle relaxation (stimulating contraction). Flavonoids are also potent inhibitors of cyclic adenosine monophosphate phosphodiesterase and calcium dependent adenosine triphosphatase[51] which prevents relaxation (stimulates contraction). However some flavonoids, inhibit cyclooxygenase enzyme [52] which can cause relaxation of muscles (inhibition). Catechins are also known to increase the level of hydrogen peroxide[53] which is involved in several downstream signalling effect[54] including inhibition of uterine smooth muscle contraction.[55] Though catechins elevate cytosolic calcium, ECG is not known to do so[56] which may also explain the inhibitory effect seen in this study. Further investigations on the role of ECG from green tea on uterine contractility are however suggested. In the same vein, kaempferol flavonoid which was also detected in green tea has been reported to increase cAMP production leading to inhibition of uterine

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Table 1: Putatively Identified Compounds in Green Tea.

S/N	Compound name	Molecular formula	Molecular weight (g/mol)	*m/z*	Rt (min)
1.	2,4(1H,3H)-Pteridinedione	$C_8H_8N_4O_2$	192.0650	$[M-H]^-$ 191.0577	1.07
2.	Quinic acid	$C_7H_{12}O_6$	192.0635	$[M-H]^-$ 191.0562	1.19
3.	2,4-Furandicarboxylic acid	$C_7H_6O_5$	170.0217	$[M-H]^-$ 169.0144	1.30
4.	Epicatechin	$C_{15}H_{14}O_6$	290.0787	$[M-H]^-$ 289.0714	2.06
5.	Quinic acid; (-)-form, 3-O-(3,4,5-Trihydroxybenzoyl)	$C_{14}H_{16}O_{10}$	344.0741	$[M-H]^-$ 343.0668	2.15
6.	1,7-Di-O-galloyl-D-sedoheptulose	$C_{21}H_{22}O_{15}$	514.0959	$[M-H]^-$ 513.0880	2.24
7.	3-O-Galloylquinic acid	$C_{14}H_{16}O_{10}$	344.0743	$[M-H]^-$ 343.0669	2.85
8.	1,2-Digalloylglucose	$C_{20}H_{20}O_{14}$	484.0853	$[M-H]^-$ 483.0767	3.49
9.	3,3',4',5,5',7-Hexahydroxyflavan; (2R,3S)-form	$C_{15}H_{14}O_7$	306.0732	$[M-H]^-$ 305.0660	3.70
10.	1-O-Caffeoyl-4-deoxyquinic acid	$C_{16}H_{18}O_8$	338.1002	$[M-H]^-$ 337.0923	3.85
11.	Luteolin- 3'-O-(3-O-Acetyl-β-D-glucuronopyranoside)	$C_{23}H_{20}O_{13}$	504.0904	$[M-H]^-$ 503.0816	4.83
12.	3-O-Galloylepigallocatechin	$C_{22}H_{18}O_{11}$	458.0847	$[M+H]^+$ 459.0920	4.85
13.	3-O-Galloylepigallocatechin	$C_{22}H_{18}O_{11}$	458.0849	$[M-H]^-$ 457.0766	5.07
14.	Myricetin 3-glucoside	$C_{21}H_{20}O_{13}$	480.0904	$[M-H]^-$ 479.0823	5.25
15.	Kaempferol 3-diglycosides; 3-O-[β-D-Galactopyranosyl-(1→2)-β-D-glucopyranoside]	$C_{27}H_{30}O_{16}$	610.1535	$[M-H]^-$ 609.1462	5.58
16.	8-C-Glucosylapigenin	$C_{21}H_{20}O_{10}$	432.1056	$[M-H]^-$ 431.0981	5.61
17.	Isotrifolin	$C_{21}H_{20}O_{12}$	464.0954	$[M-H]^-$ 463.0879	5.97
18.	3,3',4',5,7-Pentahydroxyflavan	$C_{22}H_{18}O_{10}$	442.0900	$[M-H]^-$ 441.0827	6.10
19.	3'-Galloylcatechin	$C_{22}H_{18}O_{10}$	442.0900	$[M-H]^-$ 441.0822	6.52
20.	Kaempferol 7-glucoside	$C_{21}H_{20}O_{11}$	448.1006	$[M-H]^-$ 447.0929	6.98
21.	Dihydroxyflavone	$C_{15}H_{10}O_4$	254.0579	$[M+H]^+$255.0649	11.71
22.	Dinonyl phthalate (plasticizer, artefact)	$C_{26}H_{42}O_4$	418.3083	$[M+H]^+$419.3156	14.52
23.	6'-O-Angeloy marmin	$C_{24}H_{30}O_6$	414.2042	$[M+H]^+$415.2114	17.05

Table 2: Unidentified Compounds in Green Tea Extract.

	Predicted Molecular formula	Double bond equivalence	Molecular weight (g/mol)	*m/z*	Rt (min)
24.	$C_{35}H_{24}O_2$	24.0	476.1776	$[M+H]^+$477.1867	3.87
25.	$C_{23}H_{14}N_2O_{10}$	18.0	478.0664	$[M-H]^-$ 477.0591	6.13

Double bond equivalence (DBE) indicates number of rings and double bonds in the structure where 1 ring = 1 DBE

contractility [57,58] and may also contribute to the inhibitory effect of green tea on uterine contractions in this study.

The compound, 3',4',5,7-tetrahydroxyflavone is a flavonoid also known as luteolin.[59] It is known to inhibit protein kinase C,[60] and lipoxygenase.[61] Lipoxygenase enzymes promote the breakdown of arachidonic acid into leukotrienes which interact with their receptors leading to calcium mobilization and uterine contraction.[62] Therefore inhibition of these enzymes can lead to uterine smooth muscle relaxation.[63] Luteolin is also reported to interact with gamma amino butyric acid (GABA) A receptors[64] which are well known to inhibit smooth muscle contraction[65]. Luteolin has also been reported to reduce the L-type calcium current of rat ventricular myocytes.[66] These reports suggest luteolin to be one of the active inhibitory constituents of green tea.

Quinic acid is a cyclic polyol and has been reported to produce phenolic acid derivatives.[67] Quinic acid has been reported to inhibit Nuclear Factor-kappa B (NF-κB) by increasing the production of nicotinamide and tryptophan.[68] However, an increase in nicotinamide has been shown to be positively correlated to the activity of OT in stimulating uterine contractions[69] suggesting a contraction stimulatory role for quinic acid and may not contribute to the inhibitory effect of green tea at higher concentrations in this study.

Sedoheptulose is a seven-carbon ketose sugar originally found in *Sedum spectabile* and can also be found as part of the human diet.[70] It is involved in the cyclic regeneration of d-ribulose where it acts as an intermediary compound. It is also involved in ribulose regeneration in plant photosynthesis.[70] The compound has been reported to prevent or delay diabetes onset,[71] but there appears to be no report on its involvement in uterine contractility.

Kaempferol and myricetin which were also detected in this study had been previously reported as two of the three main flavanols in tea.[72] Kaempferol has been reported to induce uterine relaxation in rats[57] and may play a role in the inhibitory effect of green tea. Though there have been no reports on myricetin on uterine contractility, myricetin and kaempferol have been shown to exert oestrogenic effect on the uterus as observed in the uterine hypertrophy induced.[73-74] Though oestrogenic effect does not directly determine effects on contractility, the similarity between myricetin and kaempferol suggests they may both exert similar effects on uterine contractility.

The interaction of the different metabolites found in green tea are seen to have potential effects on uterine contraction, while for others like kaempferol there are scientific reports to show its inhibitory effect on uterine contractility, all of which contribute to the inhibitory effect of green tea observed.

Conclusion

This study reports the inhibitory effect of green tea extract on uterine contractility. Green tea extract was shown in this study to inhibit spontaneous, oxytocin- and high KCl-induced uterine contractions. Possible interaction with prostaglandins and voltage-gated calcium channels are suggested. In addition, possible prevention of MLCK activation and interaction of green tea with IP_3 and DAG are also suggested. However these possibilities require further investigation. Several secondary metabolites were also identified in the green tea extract which was found to include pteridines, cyclitols, flavonoids and coumarins. Since the study was done using the non-pregnant uterus, it can be inferred that green tea consumption by females may contribute to easing uterine contraction which often leads to hyperalgesia during menstruation. Studies on the pregnant uterus are additionally recommended in order to gain an insight into what green tea does on the pregnant uterus.

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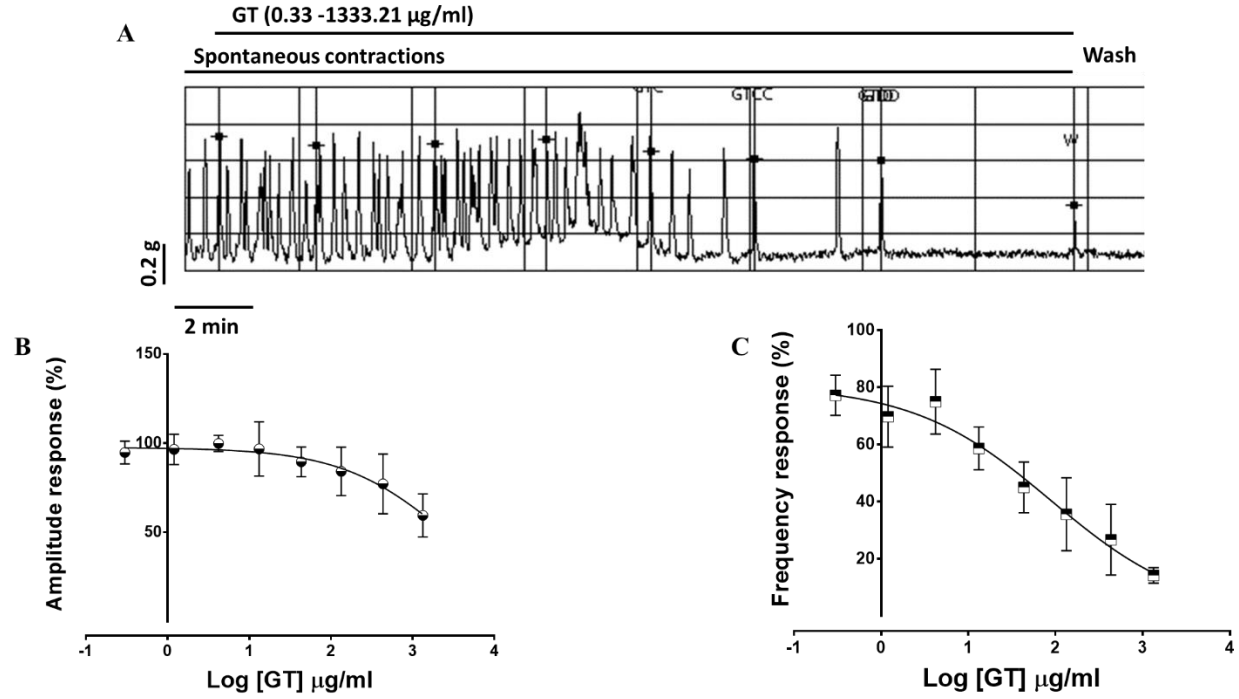

Figure 1: Effect of green tea (GT) extracts on spontaneous uterine contraction. (A). Original recording showing the effect of GT on spontaneous uterine contractility. (B). Concentration response-curve showing the effect of GT on the amplitude of spontaneous uterine contraction. (C) Concentration response-curve showing the effect of GT on the frequency of spontaneous uterine contraction. n= 5 animals.

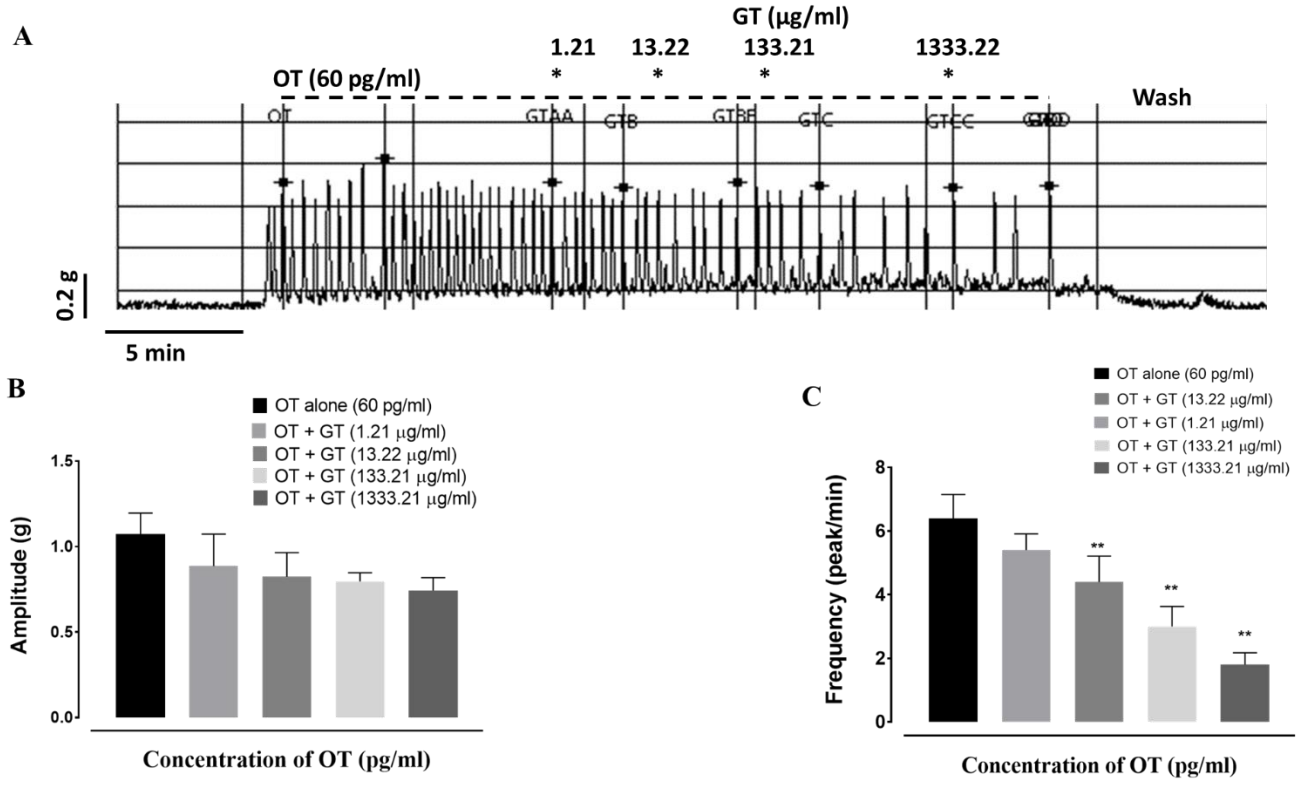

Figure 2: Effect of green tea (GT) extracts on oxytocin (OT)-induced uterine contraction. (A) Original recording showing the effect of GT on OT-induced spontaneous uterine contractility. (B) Bar graph showing the effect of GT on the amplitude of OT-induced uterine contraction (C) Bar graph showing the effect of GT on the frequency of OT-induced uterine contraction. **P > 0.001; n= 5 animals.

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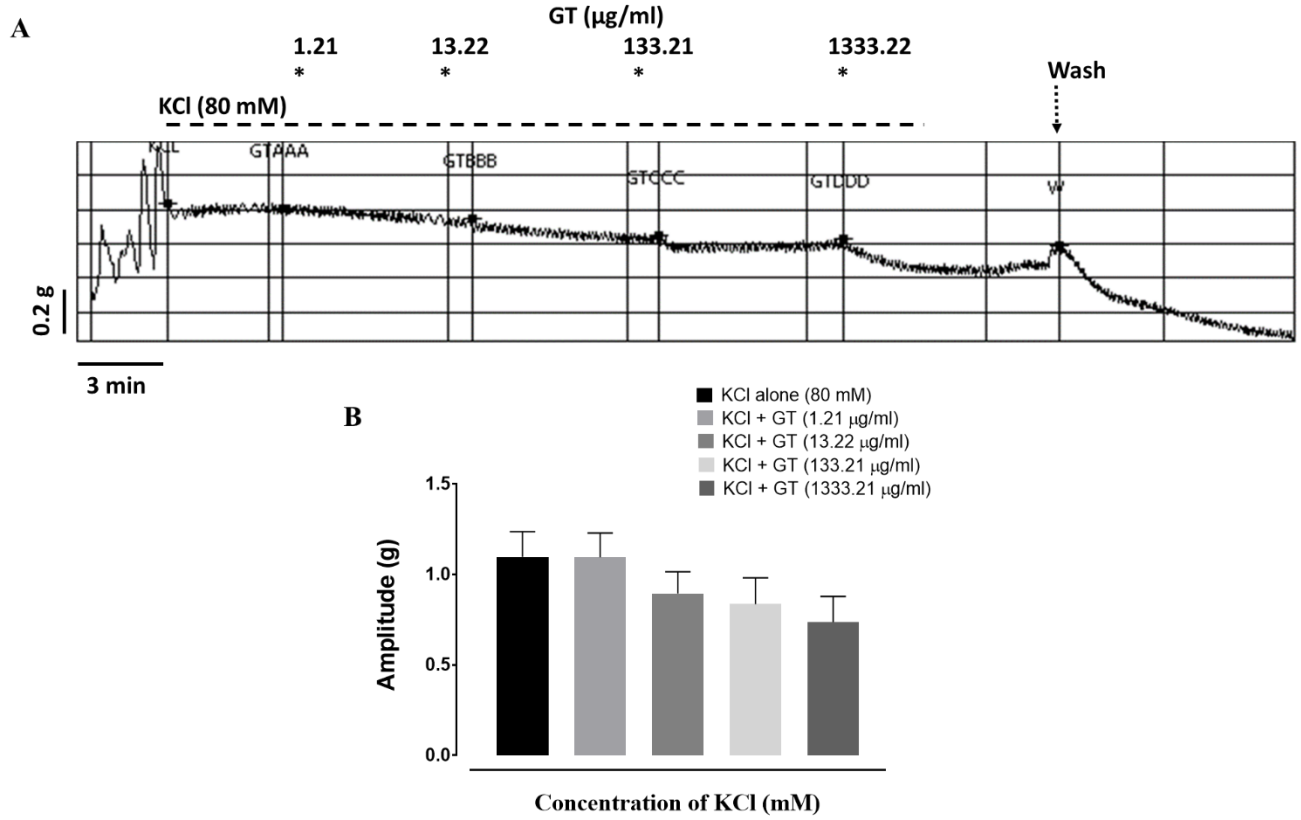

Figure 3: Effect of green tea (GT) extracts on high KCl-induced uterine contraction (80 mM). (A) Original recording showing the effect of GT on high KCl-induced uterine contraction. (B) Bar graph showing the effect of GT on the amplitude of KCl-induced uterine contraction. n= 5 animals.

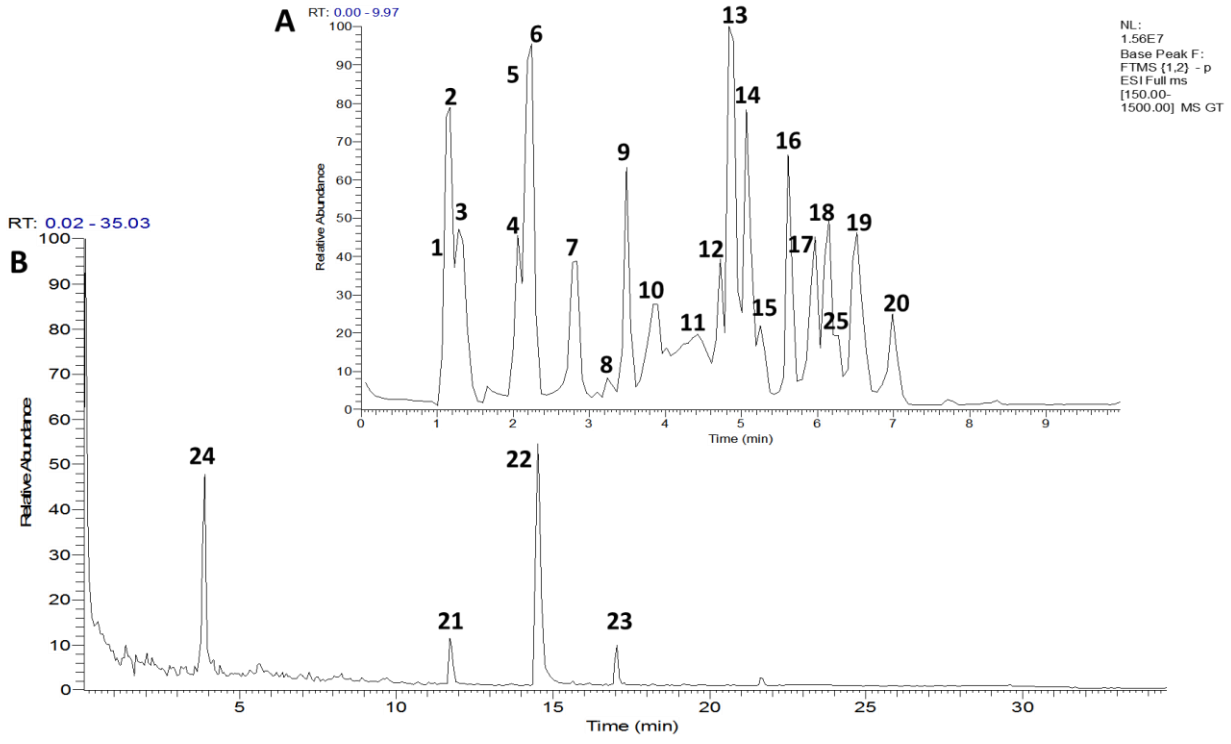

Figure 4: Total ion chromatogram for GT in **A**) negative and **B**) positive ionisation modes showing identified metabolites (**1-23**) and unidentified but detected metabolites (**24** and **25**). The identification, molecular formula, molecular weight, mass to charge ratio (m/z) and retention time in min (RT) are indicated in tables 1 and 2.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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