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Acute toxicity and uterotonic activity of aqueous extract of *Lawsonia inermis* (Lythraceae)

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ABSTRACT

To examine the folkloric claim that aqueous extract of Lawsonia inermis (AELI) induces early trimester abortion and controls postpartum haemorrhage. Acute toxicity study was conducted in mice using the method of Lorke. The uterotonic activity of AELI was evaluated in isolated myometrial strips of virgin and late pregnancy (21days) Swiss albino rats non cumulatively under 1g tension and compared with the activity of Oxytocin ($1x10^{-5}$- $2x\ 10^{-1}$IU/mL bath concentration) and the spontaneous contractions of untreated strips (control) using standard protocols. The ability of Atropine, Salbutamol and Cyproheptadine to block contractions evoked by AELI on isolated myometrial strips was also studied.AELI had an intraperitoneal LD50 of 894mg/kg in Mice and evoked statistically significant ($P<0.05$) concentration dependent contractions of the uterus of non pregnant and pregnant Wister rats with EC_{50} of 39.4 µg/mL and $2.6\ 4\ x\ 10^{-2}$ µg/mL respectively in comparison to EC_{50} of $1.3x10^{-3}$IU/mL for Oxytocin in non pregnant rat's myometrial strips. The AELI evoked contractions were resistant to atropine ($1.48x10^{-8}$M), and cyproheptadine ($1.48x10^{-8}$M) but blocked by salbutamol ($4.18x10^{-8}$M). These results support the use of Lawsonia inermis to induce first trimester abortions, prevent and treat postpartum haemorrhage in traditional medicine and suggest that uterotonic activity involving the beta-adrenergic pathway may be the mechanism.

Keywords: Postpartum hemorrhage, Phytomedicine, Median Lethal dose, Rats, Myometrial strips, Oxytocin

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INTRODUCTION

Lawsonia inermis (El-Henna) commonly known as "Lalle" by the Hausas of northwestern Nigeria is a perennial shrub native to northern Africa, Asia and Australia. It is grown extensively in Nigeria, mainly for cosmetic purposes. The leave extracts have been shown to possess antibacterial [1] antifungal [2] antipyretic and analgesic [3] antioxidant and immunomodulation [4] activities. The aqueous leaves extract of *Lawsonia inermis*(AELI) is used as folk medicine among the Hausa tribes of north-western Nigeria against amoebiasis, headache, jaundice, leprosy , post coital contraception, early trimester abortion and to stop uterine bleeding, especially in the immediate postpartum period (Musa A.S, Umar B.A, Yusuf S.S, Herbal medicine practitioners, Sokoto, Personal Communication). Postpartum hemorrhage (PPH) is a significant cause of maternal mortality and is implicated in about a quarter of maternal deaths worldwide [5]Of the various causes of PPH, uterine atony is the most common. The common drug options for prophylaxis and management of PPH is a short list of three uterotonic drugs that includes oxytocin, ergometrin and misoprostol. These drugs are only available in hospital settings. In developing economies, like Nigeria, 80 percent of the inhabitants depend on herbs for treating their illnesses [6]. The safety and efficacy of traditional medicine is therefore an important concern for both health authorities and the general public. The aim of this study was, therefore, to obtain preliminary evaluation of the safety of AELI and to examine the folkloric claims of effect of this extract on reproductive health.

MATERIALS AND METHODS

Animals

Virgin female Swiss mice weighing 18-22g, Male Wister rats and virgin female Wister rats weighing 140-250g maintained at the animal facility of the National Institute for Pharmaceutical Research and Development, Abuja, were used. The care of animals conformed to the guidelines for the care and use of experimental animals of the US National Research Council [7]

Drugs

Oxytocin, Atropine, Salbutamol (Sigma Chemical company, USA), and Cyproheptadine (Merck, Sharp and Dohme, Pakistan).

Collection of Plant materials

The leaves of *Lawsonia inermis* were harvested by two of the authors(BSO and BI) in June from growing plants in a cultivar at Kara, Sokoto, Nigeria. They were identified by I.Muazzam and A. Ohaeri of the Herbarium Unit, National institute for Pharmaceutical Research and Development (NIPRD), Abuja where a voucher specimen (accession number BA112) has been deposited.

Preparation of plant extract

Leaves were washed, air-dried to constant weight, cut into small pieces and 500g was then simmered in deionised water for 24 hours. This extraction process closely resembled the ethnic technique of preparation. The aqueous filtrate was lyophilized, weighed and the extract yield was calculated relative to the dry starting material (3.9% w/w). The lyophilized crude extract was resolubilized in deionised water to desired concentrations. The herbal extract was consequently evaluated as phytomedicines according to the World Health Organization designation of herbal medicine [8]

Phytochemical screening of AELI

Phytochemical screening for major constituents of AELI was designed and conducted according to standard qualitative and quantitative methods as described by Trease and Evans [9] and El-Olemmy et al [10]. AELI was tested for alkaloids, flavonoids, Tannins, glycosides, saponins and essential oils.

Assessment of acute toxicity of AELI

Acute toxicity evaluation was designed and conducted according to the method of Lorke [11]. Briefly, three doses in log progression (i.e. 10mg/kg, 100mg/kg,1000mg/kg) were administered to 4 mice each and 100% death was observed at 1000mg /kg while no death occurred at the lower doses. Subsequently, 100 female mice were randomly allocated into five groups of 4 mice per dose levels (i.e. 20 mice per dose level) of 100mg/kg, 200mg/kg, 400mg/kg, 800mg/kg and 1000mg/kg intraperitoneal (i.p.) AELI respectively. After i.p. administration of AELI, animals were observed every 15 minutes for the first 4 hours, then every 2 hours for the next 8 hours, then daily for 14 days.

Evaluation of uterotonic activity AELI

In separate experiments, using 20mL jacketed Ugo Basile organ bath set up with isometric transducer model 7004 and Unirecorder Model 7050, uterotonic activity of AELI on isolated 16mm myometrial horn strips of virgin female rat (pre-treated with 0.1mg/kg stilbesterol 24hrs earlier) and late pregnant rat (day 21; day when vaginal smear was found positive for sperm was take as day 0) bathed with De Jalon's solution with the following composition (mmol) NaCl 154.0, KCl 5.6, $CaCl_2$ 0.5, NaHCO3 6.0 and glucose 2.8. , aerated with 5% carbon dioxide in Oxygen, was studied non cumulatively under 1g tension and compared with the activity of Oxytocin (1x10-5- 2x 10-1IU/mL bath concentration) and spontaneous contractions of untreated strips (control) using standard protocols. The ability of Atropine, Salbutamol and Cyproheptadine to modify contractions evoked by AELI on isolated myometrial strips was also studied using standard protocols.

Assessment of effect of AELI on contraction of non pregnant rat's myometrial strip.

In this experiment, the organ baths were maintained at a temperature of 37±1 °C. Each myometrial strip preparation (n=8 for each dose) was allowed to stabilize for 60 min. Normal myometrial activity was recorded as the baseline control. This was followed by testing for the effects of 1.5 x 10^{-1} IU/mL of oxytocin and increasing concentration of AELI. Each strip was used only once with 1 minute drug contact time.

Assessment of effect of AELI versus Oxytocin on non pregnant rat's myometrial strips

In this experiment, the bath temperature was maintained at 26 ±1 ^{0}C which inhibited spontaneous contractility. Each myometrial strip preparation (n=4) was allowed to stabilize for 60 min. This was followed by testing for the effects of Oxytocin (Sigma USA), LIAE, and then Oxytocin again in that order. A 5 min dose cycle consisting of 1 min contact time, 1 min for rinsing and 3min recovery time was used.

Assessment of effect of antagonists on AELI induced contraction of non pregnant rat's uterus.

In this experiment, the bath temperature was maintained at 26 ±1 ^{0}C which inhibited spontaneous contractility. Each myometrial strip from non pregnant rat was allowed to stabilize for 60 min. Control responses of myometrial strips to sub-maximal concentration (700µg/mL) of the extract were obtained. Atropine ($1.48x10^{-8}$M) , Cyproheptadine ($1.48x10^{-8}$M) , and Salbutamol ($4.18x10^{-8}$M) was then added singly and allowed to bath the tissue for 10 minutes before adding the extract. Each experiment was completed by obtaining a response to the extract again after washing. Each antagonist was tested on separate preparations (n=4).

Assessment of effect of AELI on late pregnant rat's myometrial strips

In this experiment, the organ baths were maintained at a temperature of 37±1 °C. Each myometrial strip preparation (n=8 for each dose) was allowed to stabilize for 60 min. Normal myometrial activity was recorded as the baseline control. This was followed by testing for the effects of 1.5×10^{-1} IU/mL of oxytocin and increasing concentration of AELI. Each strip was used only once with 1 minute drug contact time.

Statistical Analysis

Student's t-test was used for pair wise comparison. Dose response curves, $E_{maximum}$ (concentration causing maximum response) and EC_{50} (concentration causing 50% of the maximum response) were determined by nonlinear regression using the least squares method. Concentrations were log transformed prior to determination of potencies (EC50) and efficacies ($EC_{maximum}$). Statistical significance was set at $p<0.05$. Data were analyzed with STATS version 1.1(Decision Analyst Inc., USA) and GraphPad Prism version 5.01 (GraphPad Software Inc., USA)

RESULTS

Phytochemistry of AELI

AELI was found to contain Tannins (1.3%w/w), Alkaloids (0.7%w/w), Glycosides (0.53%w/w) and saponins (0.65% w/w). It also contained traces of reducing sugars and steroids but no flavonoids.

Acute toxicity of AELI

AELI revealed an intraperitoneal LD50 of 894mg/kg in Mice (Table 1). No evidence of toxicity was observed at doses up to 800mg/Kg. All animals given 1000mg/kg became sluggish within 2hours, fell into sleep within 6hours and died within 12 hours of drug administration. No animal had convulsion nor diarrhoea.

Effect of AELI on contractions of non pregnant rat's myometrial strip.

At concentrations of 100 -1600µg/mL, AELI significantly ($P<0.05$) augmented the force of spontaneous contractions of myometrial strips from non pregnant Wister rats with $EC_{maximum}$ of 799µg/mL and EC_{50} of 39.4 µg/mL but significantly augmented the frequency of contractions only at concentrations of 400-1600µg/mL(Table 2)

Effect of AELI versus Oxytocin on non pregnant rat's myometrial strips

AELI evoked significant dose dependent myometrial contraction of quiescent myometrial strips from non pregnant rat's uterus with $EC_{maximum}$ of 713 µg/mL and EC_{50} of 29 µg/mL. In the same setup, The $EC_{maximum}$ and EC_{50} of Oxytocin were 2×10^{-1}IU/mL and 1.3×10^{-3}IU/mL respectively. The maximum force of contraction of 5.94 ± 0.05 g was caused by 713 µg/mL of AELI, while a maximum force of contraction of 5.61 ± 0.02 g was caused by 2×10^{-1} IU/mL of Oxytocin (The intrinsic activity of the extract was therefore taken as 1.06)(Figure 1)

Effect of antagonists on AELI evoked contractions of non pregnant rat's uterus.

AELI evoked contractions were resistant to atropine (1.48x10-8M), and cyproheptadine (1.48x10-8M) but completely antagonised by 4.18x10-8M salbutamol.

Effect of AELI on contractions of late pregnant rat's myometrial strip.

At concentrations of 50 -800µg/mL , AELI significantly ($P<0.05$) augmented both the force and frequency of spontaneous contractions of myometrial strips from late pregnant Wister rats with $EC_{maximum}$ of 200µg/mL and EC_{50} of $2.6\ 4 \times 10^{-2}$ µg/mL (Table 3)

DISCUSSION AND CONCLUSION

AELI appears to have an Oxytocin like effect on non pregnant rat's myometrial strips but is both more potent and has higher efficacy on pregnant rat's myometrial strips which suggests that the pregnant milieu augments the uterotonic pathway of AELI. Unlike Oxytocin, AELI is active after oral administration which suggests that the active component is not a peptide. The lack of effect of atropine on the uterotonic effect of AELI suggests that AELI may not be acting through muscarinic . Also, the lack of effect of cyproheptadine on AELI induced contractions suggests that the uterotonic activities of AELI may not involve histerminergic , cholinergic or serotonergic receptors and might not involve calcium channel openings . The ability of salbutamol to block AELI evoked contractions implies that physiologic antagonism by beta-adrenergic mechanism is possible against the

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uterotonic effect of AELI. The oral acute toxicity of AELI in mice is reassuring. The LD50 of 894mg/kg /i.p. compares favourably with the subcutaneous (no data exists for i.p.) LD50 0f Oxytocin in mice (514mg/kg) [12] and that of other uterotonic ethno medicinal plant extracts like *Brysocarpus coccineus (*547mg/kg/oral*)* [13]. These results seem to support the use of *Lawsonia inermis* to induce first trimester abortions prevent and treat postpartum haemorrhage in traditional medicine.

Table 1. Determination of LD_{50} of AELI

Dose(mg/kg)		Mortality per 4 animals					Total Mortality per 20 animals
	Group	1	2	3	4	5	
100		0/4	0/4	0/4	0/4	0/4	0/20
200		0/4	0/4	0/4	0/4	0/4	0/20
400		0/4	0/4	0/4	0/4	0/4	0/20
800		0/4	0/4	0/4	0/4	0/4	0/20
1000		4/4	4/4	4/4	4/4	4/4	20/20

LD_{50}= √ (800x1000) =894mg/kg i.p. in Wister rats

Table 2. Effect of aqueous extract of *Lawsonia inermis* (AELI) on contractions of non pregnant rat's myometrial strip

Dose levels AELI in (µg/ml) and Oxytocin in (IU/mL)	Force of contraction (g tension)			Frequency (contractions/minute)		
	Baseline	Plus Drug	P-Value	Baseline	Plus Drug	P-value
25 AELI	4.05±0.55	4.30±0.58	0.55	1.04±0.12	1.17±0.10	0.15
50 AELI	4.05±0.32	4.50±0.52	0.28	1.17±0.10	1.34±0.19	0.16
100 AELI	4.35±0.32	5.15±0.43	0.034	1.25±0.14	1.17±0.10	0.39
200 AELI	3.95±0.38	5.60±0.40	0.001	1.13±0.22	1.17±0.10	0.75
400 AELI	4.60±0.23	5.85±0.09	0.0001	0.92±0.24	1.34±0.19	0.034
800 AELI	4.65±0.03	5.95±0.03	0.0001	1.00±0.00	1.34±0.19	0.011
1600 AELI	3.90±0.29	5.60±0.06	0.0001	0.82±0.11	1.29±0.02	0.0002
1.5 Oxytocin	3.95±0.22	4.6±0.05	0.0001	1.03±0.11	0.63±0.16	0.0001

Results are expressed as Mean± SEM of 8 observations. Data was analysed using STATS. Pair wise comparison was done using paired t-test. $P<0.05$ was considered significant. No correction for multiplicity was done because baseline contractions versus contractions after AELI was applied were the comparison of interest at each dose level.

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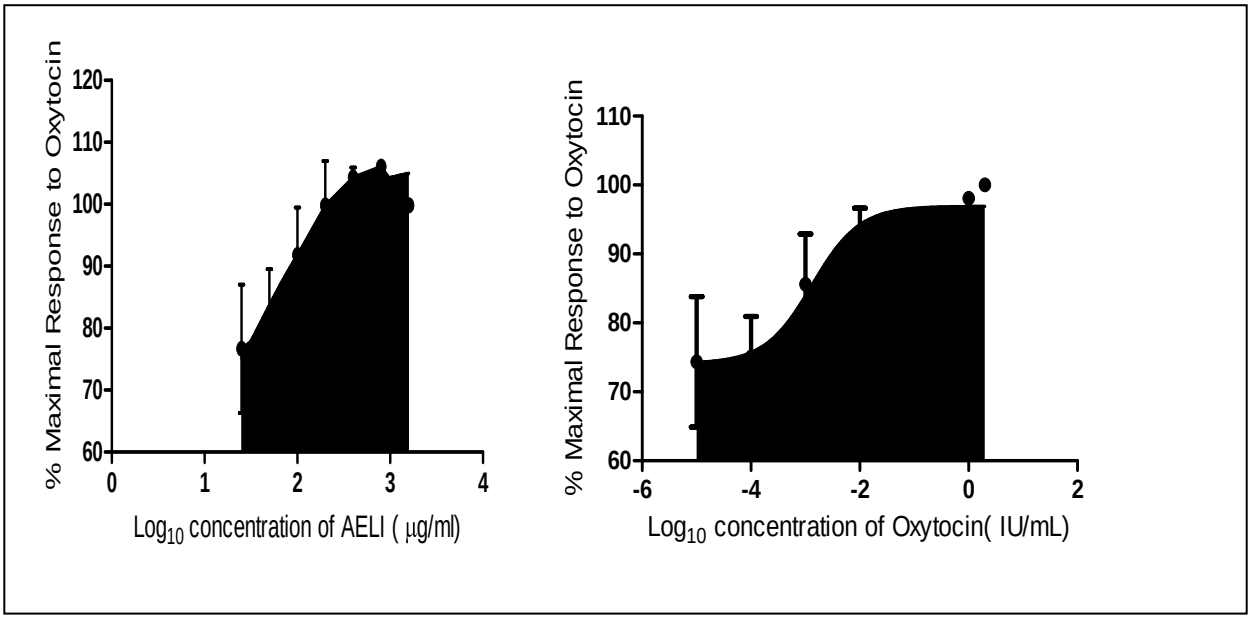
Table 3. Effect of *Lawsonia inermis* aqueous extract (AELI) on late pregnant rat's myometrial contraction

Dose levels AELI in (µg/ml) and Oxytocin in (IU/mL)	Force of contraction (g tension)			Frequency (contractions/minute)		
	Baseline	Plus Drug	P-Value	Baseline	Plus Drug	P-value
50 AELI	0.55±0.03	1.25±0.38	0.01	0.80±0.12	1.24±0.05	0.0005
100 AELI	0.80±0.17	3.00±0.23	0.0001	0.94±0.15	1.07±0.04	0.045
200 AELI	0.80±0.23	6.20±1.15	0.0001	0.84±0.10	0.98±0.07	0.006
400 AELI	1.90±0.64	5.25±1.59	0.008	0.64±0.02	0.95±0.03	0.0001
800 AELI	1.60±0.52	5.05±1.70	0.008	1.00±0.00	0.80±0.00	0.0001
1.5 Oxytocin	0.95±0.23	3.66±0.21	0.0001	0.91±0.14	0.52±0.06	0.0001

Results are expressed as Mean± SEM of 8 observations. Data was analysed using STATS. Pair wise comparison was done using paired t-test. P<0.05 was considered significant. No correction for multiplicity was done because baseline contractions versus contractions after AELI was applied were the comparison of interest at each dose level.

Figure 1. Effect of AELI versus Oxytocin on non pregnant rat's myometrial strips. Data was analysed using GraphPad Prism. The EC_{50} of LIAE was 39.4µg/mL while the EC_{50} of Oxytocin was $1.3x10^{-3}$IU/mL.



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REFERENCES

[1] Ali NA, Jülich WD, Kusnick C, Lindequist U. J Ethnopharmacol 2001; 74:173-9.

[2] Singh VK, Pandey DK. Hindustan Antibiot Bull 1989; 31:32-35
[3] Ali BH, Bashir AK, Tanir MO. Pharmacology 1995;51: 356-363
[4] Mikhaeil BR, Badria FA, Maatooq GT, Amer MM. Naturforsch C 2004; 59 : 468-76
[5] AbouZahr C. Antepartum and postpartum hemorrhage. In: Murray CJ, Lopez AD, eds. Health dimensions of sex and reproduction: the global burden of sexually transmitted diseases, HIV, maternal conditions, perinatal disorders, and congenital abnormalities. Boston, MA: Harvard University Press, 1998; 172–4
[6] Hostettmann K, Marston A. Phytochemistry Reviews 2002; 1: 275- 285
[7] National Research Council. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Washington DC, National Academy Press,1996; 117-120
[8] WHO . (2008).Traditional medicine . WHO/Fact sheet N°134. Available at: http://www.who.int/mediacentre/factsheets/fs134/en/ Accessed on 7 July 2009
[9] Trease GE, Evans MC. Textbook of Pharmacognosy. London: Bailliere Tindall, 1983;343–383
[10] El-Olemmy MM, Al-Muhtadi AS, Affifi AA. Experimental Phytochemistry. A laboratory Manual(Ed). Riyadh, Saudi Arabia: King Saudi University Press, 1994;14-15.
[11] Lorke D. Arch Toxicol 1983;54:275-287.
[12] TEVA USA . Oxytocin Injection, USP, Material Safety Data Sheet. Available at : http://www.tevausa.com/assets/base/products/msds/Oxytocin_MSDS.pdf Accessed on 29 November 2009
[13] Amos S, Binda L, Kunle OF, Wambebe C, Gamaniel K. Pharm Biol 2002; 40: 33-38

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