

# Effect of *Asparagus racemosus* on selected female reproductive parameters using Wistar rat model



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## ABSTRACT

Dysmenorrhea is a major cause of female morbidity globally. *Asparagus racemosus* (ASP) is traditionally used to manage dysmenorrhea in Nakuru, Kenya. This study evaluates the effect of *Asparagus racemosus* on female reproductive parameters; estrus cyclicity, mating success, gestation length, litter size and dysmenorrhea. The effect of *Asparagus racemosus* and ibuprofen on isolated uterine strips was evaluated using six non-pregnant rats. The uterine strips were exposed to serial extract concentrations (20, 40, 80, 160 mg/ml) and 20 mg/ml ibuprofen was used as a positive control.

There was significant increase of proestrus phase ( $P < 0.001$ ) and a subsequent significant reduction in the metestrus ( $P < 0.01$ ) and

diestrus ( $P < 0.05$ ). No significant difference in mating success and gestation length, however ibuprofen caused significant disruption of gestation length ( $P < 0.05$ ). The treated groups produced higher number of pups compared to controls. The plant extract caused a dose dependent significant reduction in uterine force of contraction by (-0.15%, -5.13%, -7.97%, -19.55 %) at 20, 40, 80 and 160 mg/ml respectively. The plant extract also caused a significant decline in frequency of uterine contraction (-5.99%; -9.61%; -16.76% and -25.21%) The extract caused no mortality even at the limit dose; 5000 mg/kg. *Asparagus racemosus* reduces uterine force and frequency of contraction. This is probably the reason for its traditional use in dysmenorrhea management.

**Key words:** *Asparagus racemosus*, estrus cyclicity, mating success, litter size, uterine contractility

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## INTRODUCTION

Dysmenorrhea is the major gynecological problem among women of reproductive age globally (Ozgoli et al., 2009; Proctor and Farquhar, 2006). However, it is usually under-diagnosed and under-treated and most women regard the pain as a normal condition of the menstrual cycle (Wong, 2011). Dysmenorrhea can either be primary or secondary (Proctor and Farquhar, 2006). Primary dysmenorrhea is the painful cramping of the lower abdomen without any identifiable pelvic pathological condition just before or during menses (De Sanctis et al., 2016). It is associated with insomnia, mood disturbances and reduced daily activities (Allen and Lam, 2012). It is widely accepted to be caused by over-production of uterine prostaglandins (PG) leading to hyper-contraction of the myometrium, ischemia and hypoxia (Dawood, 1987). Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and Oral contraceptives (OCPs) the common conventional treatments cause a plethora of side effects (Proctor et al., 2001). Therefore the search for alternative remedies including medicinal plants is necessary.

*Asparagus racemosus* of family *Asparagaceae* has been widely used in the management of dysmenorrhea. The major bioactive components present

in the root of *Asparagus racemosus* are the steroidal saponins, flavonoids, isoflavonoids, glycosides, minerals like Calcium, Magnesium, Iron, Phosphorus and vitamins A, B1, B2, C, E (Shaha and Bellankimath., 2017; Joshi, 2016). Phytoestrogenic activity of *Asparagus racemosus* is due to the steroidal saponins which enables estrogen hormone balance (Joshi, 2016). The plant has been used to alleviate menstrual disorders ranging from premenstrual syndrome (Kinage and Chaudhari, 2016). The role of *Asparagus racemosus* in alleviating dysmenorrhea is not clear and the literature is very scanty. This study was aimed at elucidating effects of *Asparagus racemosus* on related estrous cyclicity, pregnancy outcome and its effect on isolated myometrial quiescence.

## MATERIALS AND METHODS

### Extract preparation

The study was carried in Olenguruone area in Nakuru County, Kenya. The plant was collected in January 2019, washed and allowed to dry, then transported to the Department of Veterinary Anatomy and Physiology, University of Nairobi. The dried roots were chopped into smaller pieces using a knife, ground into fine powder. The voucher specimen of

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the plant was deposited in the School of Biological Sciences herbarium (University of Nairobi) and assigned specimen number (RI2019/08). Three hundred grams of the powder was put in 3litres of boiling distilled water for about 30 minutes. The mixture was allowed to cool, filtered and dried in an oven at 45°C. The percentage yield was calculated (6.67%) and stored in a refrigerator (-20°C) until use. Kenyan *Asparagus racemosus* preliminary phytochemical screening was carried out following the method used by Periyasamy and Mahalingam (2010) to screen for presence of saponins, alkaloids, flavonoids, tannins, terpenoids and glycosides.

### Experimental Animals

Female albino Wistar rats 5-6 weeks old were used. They were purchased from Biochemistry Department, University of Nairobi. Animals were housed in the Department of Veterinary Anatomy and Physiology animal house. Wood shavings were used as beddings and were replaced every other day. Animals were maintained under standard environmental conditions of 12 hour light and 12 dark cycles, temperatures of 24-25°C. They were provided with adequate rat-pellets from Bellmill feeds limited (Kenya) and water *ad-libitum*. Animal welfare and Ethics clearance was sought from the Faculty of Veterinary Medicine Biosafety and Animal Ethics committee (FVM/BAUEC/2019/186). Anti-plagiarism report (ID 1154321540) was carried out by University of Nairobi library.

### Experimental protocols

#### Acute oral toxicity

A total of 12 nulliparous non pregnant female rats were used. They were divided into groups of 3 and the experiment was carried out following the OECD guideline 423. Their weights were recorded on day 0, 1, 7 and 14. Acute oral toxicity was done to determine lethal dose (LD50) and safety of the plant extract.

#### Cyclicity, mating success, gestation length and litter size

A total of 20 nulliparous non pregnant normal cyclic female Wistar rats aged 6 weeks old were used. Estrus cyclicity was monitored daily 9-10am for 20 days to ensure normalcy. The rats were divided into 4 groups of 5 rats each. They received *Asparagus racemosus* extract every other day for 14 days. Group I (600 mg/kg) and group II (300 mg/kg) *Asparagus racemosus* root extract respectively, group III (20 mg/kg) ibuprofen (positive control), group IV (0.5ml) physiological saline (negative control). Vaginal flush was done daily between 9 to

10 am for all the rats and estrus stages recorded. After the 14 days, males of proven fertility were introduced into the cages. Vaginal swabbing and microscopy was done daily between 8-9 am to determine presence or absence of spermatozoa. The day spermatozoa was seen was recorded as day 1 of pregnancy, the female was put in a separate cage and monitored daily by weighing until they littered. The litter size and gestational length for all rats was recorded.

#### Preparation of uterine strips for uterine contractility

A total of 6 nulliparous non pregnant female rats aged 4 months old were used. They received 2 mg/kg b/w intra peritoneal stilboestral injection 24 hours before the onset of the experiment. Immediately thereafter, the uterine horns were carefully harvested, excised of connective tissues and placed in a dish of de Jalon's solution. De Jalon solution was a mixture of sodium chloride (9 grams), sodium hydrogen carbonate (2.1 grams), glucose (0.5 grams), potassium chloride (0.402 grams), calcium chloride (0.24 grams), sucrose (4.5 grams), sodium dihydrogen phosphate (0.142 grams) in a litre of distilled water. The solution was maintained at 35± 0.5°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Uterine strip, 2cm in length was cut and mounted vertically within the organ bath with one end fixed and the other one attached to an isotonic force transducer (ML500/A, AD Instrument) coupled with Power Lab data acquisition system (Power Lab 8/30). The force of contraction (grams) and frequency of contraction (beats per minute) were recorded and analyzed using Chart5 software for windows. One uterine strip at a time was mounted in the organ bath. The uterine strip was stabilizing for approximately 20minutes, then negative control readings recorded for 10 minutes. Uterine strips were thereafter exposed to 20, 40, 80 and 160 mg/ml of the extract (Kaingu et al., 2011); beginning with lowest concentration (20mg/ml). The uterine strip was rinsed 3 times with de Jalon solution, allowed 20 minutes recovery; then exposed to 40mg/ml *Asparagus racemosus* root extract. Contractions were recorded for 10 minutes. The procedure was repeated until the strip had been exposed to all the extract concentrations. The process was repeated six times using fresh uterine strips. The effect of *Asparagus racemosus* root extract on the frequency (rate) of uterine contraction as well as contraction strength (force) was recorded. The frequency was given by the event counts determined by the number of peaks in the selected region. The strength was given by the means of the data points

in the selected region over a period of 10 minutes. The treatment readings were compared with the positive and negative control recordings.

### Data analysis

Data collected was entered into MS- excel data sheet and cleaned. The results were recorded as Mean  $\pm$  SEM. Data was analyzed using SPSS and graphs were generated using Graphpad Prism version 8. Descriptive statistics was carried out on the outcome variables. One-way ANOVA followed by Bonferroni post hoc test were used to compare the outcomes between groups. P-values ( $P < 0.05$ ;  $P < 0.01$ ;  $P < 0.001$ ) were considered significant.

## RESULTS

### Phytochemistry and acute oral toxicity of *Asparagus racemosus* root extract

Kenyan *Asparagus racemosus* root extract phytochemistry showed presence of saponins, flavonoids, terpenoids and glycosides. Alkaloids and tannins were absent. Animals used for the acute oral toxicity study showed no significant difference in their weight gain compared to the control group ( $P > 0.05$ ). *Asparagus racemosus* root extract did not cause any mortality even at the 5000 mg/kg.

### Effect of *Asparagus racemosus* aqueous root extract on estrus cyclicity, mating success, gestation length and litter size

Estrus cycle disruption was observed in the high (600 mg/kg) and low (300 mg/kg) dose group

compared to the negative control. There was a significant difference ( $P < 0.05$ ) in the proestrus phase within the treated group compared to control. Metestrus and diestrus phases were significantly ( $P < 0.01$  and  $P < 0.05$ ) disrupted respectively compared to control group. There was no significant difference in the estrus phase compared to the control groups ( $P > 0.05$ ). All rats treated with ibuprofen had increased appearance of frequency of proestrus with subsequent reduction in frequency of metestrus and diestrus phase.

### Mating success, conception, gestation and litter size

*Asparagus racemosus* aqueous root extract at 600 mg/kg and 300 mg/kg caused no significant difference to mating success (100% success) compared to the control. All rats conceived (100%) compared to negative (80%) and positive control (40%). *Asparagus racemosus* root extract did not cause a significant difference in gestation length compared to the negative control. However, ibuprofen caused a significant difference in the gestation length ( $P < 0.05$ ) compared to treatment groups (Table 2). There was no significant difference in the litter size for both treatment groups compared to the negative and positive controls (Table 2). Only 2 rats in the ibuprofen treated group littered out of the 5.

### Effect of *Asparagus racemosus* aqueous extract on isolated uterine strip contraction

Percentage contraction was obtained by the formula (% contraction = force of contraction after

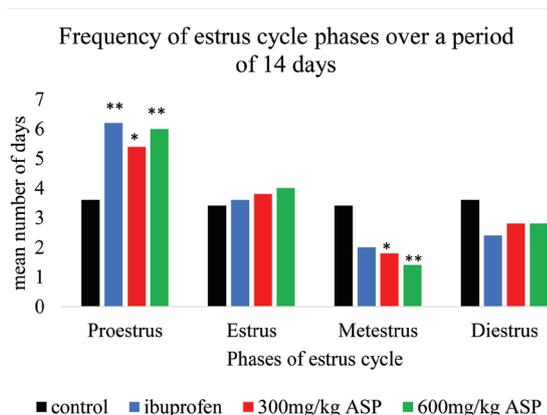
**Table 1** Effect of *Asparagus racemosus* aqueous root extract on estrus cyclicity.

Treatment	Proestrus	Estrus	Metestrus	Diestrus
Negative control	3.60 $\pm$ 0.4	3.4 $\pm$ 0.245	3.4 $\pm$ 0.4	3.6 $\pm$ 0.245
600 mg/kg ASP	6.00 $\pm$ 0.316**	4.00 $\pm$ 0.316	1.4 $\pm$ 0.4**	2.8 $\pm$ 0.2
300 mg/kg ASP	5.40 $\pm$ 0.6*	3.8 $\pm$ 0.2	1.8 $\pm$ 0.374*	2.8 $\pm$ 0.374
Positive control	6.20 $\pm$ 0.2**	3.60 $\pm$ 0.4	2.00 $\pm$ 0.316	2.40 $\pm$ 0.245*
P-value	0.001***	0.543	0.009**	0.044*

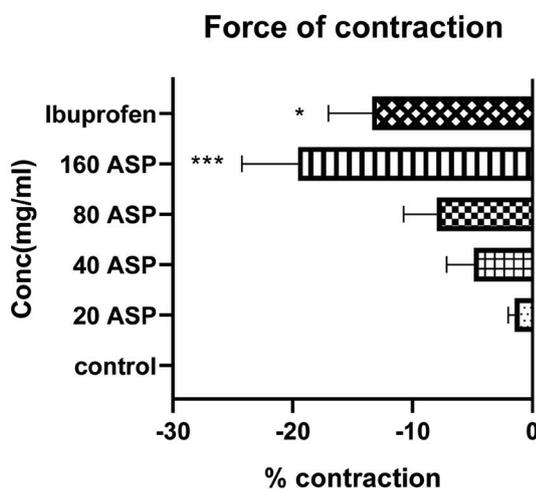
Estrus cycle was monitored for 14 days. Values represent Mean  $\pm$  SEM for the frequency of appearance of the various phases. Values with superscripts \*, \*\*, \*\*\* have a significant difference at ( $P < 0.05$ ;  $P < 0.001$ ;  $P < 0.0001$ ) respectively compared to the negative control.

**Table 2** Effect of *Asparagus racemosus* on mating success, gestation length and litter size

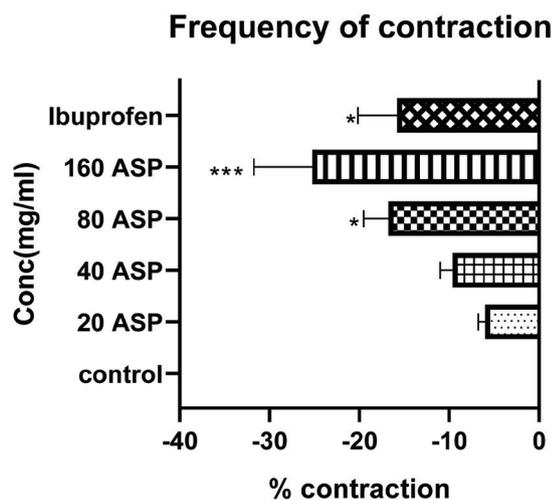
Treatment	Mating success (%)	Conception rate (%)	Gestation length (number)	Litter size (number)
Negative control	100%	80%	21.75 $\pm$ 0.25	9.75 $\pm$ 0.5
600 mg/kg ASP	100%	100%	21.80 $\pm$ 0.2*	10.60 $\pm$ 0.4
300 mg/kg ASP	100%	100%	21.80 $\pm$ 0.2*	9.80 $\pm$ 0.9
Positive control	100%	40%	23.00 $\pm$ 0.0*	7.50 $\pm$ 1.5
P-value			0.026*	0.144



**Figure 1** The effect of *Asparagus racemosus* root extract and ibuprofen on estrus cyclicity for a period of 14 days (n=5)



**Figure 2** The effect of *Asparagus racemosus* and Ibuprofen on force of uterine contraction



**Figure 3** The effect of *Asparagus racemosus* and ibuprofen on frequency of uterine contraction

treatment - control contraction, divided by control contraction  $\times 100$ ). There is significant difference in force of contraction compared to control ( $P < 0.0001$ ). 20, 40, 80, 160 mg/ml *Asparagus racemosus* root extract reduced the percentage force of contraction by -0.15%; -4.87%; -7.97% and -19.55% respectively. The decrease in force of contraction was in a dose dependent manner. Upon addition of Ibuprofen, the percentage decrease in uterine force of contraction was -13.38% compared to the negative control.

The frequency of uterine contraction was similarly reduced significantly ( $P < 0.0001$ ). 20, 40, 80, 160 mg/ml *Asparagus racemosus* root extract, reduced the frequency of uterine contraction by -5.99%, -9.61%, -16.76% and -25.21% respectively. A significant difference in the frequency of uterine contraction was at 80 and 160 mg/ml ( $P < 0.05$  and  $P < 0.0001$ ) respectively compared to the negative control. Ibuprofen caused a percentage decrease in the frequency of uterine contraction by -15.78%.

## DISCUSSION

*Asparagus racemosus* root extract is used by traditional herbalists in Nakuru County, Kenya to alleviate/ manage dysmenorrhea. Preliminary phytochemistry of Kenyan *Asparagus racemosus* revealed presence of saponins, flavonoids, glycosides and terpenoids. Glycosides and flavonoids (Telafo *et al.*, 2012) promotes fertility. *Justicia insularis* flavonoids and glycosides improved fertility in female rats by inducing ovarian steroidogenesis and folliculogenesis (Tefalo *et al.*, 2012). *Solanum torvum* used for its analgesic properties contain flavonoids, saponins, tannins and glycosides (Ndebia *et al.*, 2007). These estrogenic compounds could probably be the reason for its use to relieve pain. Khatami *et al.*, 2017 also reported presence of flavonoids and glycosides the main agents for analgesic effect on the uterine muscle in Chamomile flowers which is used in treatment of dysmenorrhea. Acute oral toxicity studies caused no mortality even at 5000 mg/kg. Kumar *et al.*, 2010 reported similar results where *Asparagus racemosus* caused no mortality at 3200 mg/kg.

## Estrus cyclicity

*Asparagus racemosus* root extract caused an increased appearance frequency of proestrus and estrus and subsequent reduction in appearance of metestrus and diestrus phases at 300 and 600 mg/kg respectively. This could be due to estrogenic properties of *Asparagus racemosus*. Estrogen levels increase during proestrus phase and are beneficial to development and maturation of follicles (Satue and

Gardon 2013). In this study, frequency of proestrus and estrus increased. Estradiol causes endometrial thickening and proliferation in preparation for implantation. Effect of *Asparagus racemosus* could be at pituitary or gonadal level. In late luteal phase of non-pregnant animals, uterine production of prostaglandin PGF2 $\alpha$  increases. PGF2 $\alpha$  is transported to the corpus luteum (Johnson, 2002) and initiates luteolysis. The resultant reduction in progesterone and elevated PGF2 $\alpha$  leads to dysmenorrhea. A decrease in appearance frequency of metestrus and diestrus phase probably might be caused by a reduction in of PGF2 $\alpha$ . Exploration of this theory might be useful in generating a novel analgesic drug. Studies have shown that during late luteal phase (late stages of metestrus and diestrus) the levels of estradiol starts to increase and progesterone decrease (Walmer et al, 1992). This is when dysmenorrhea starts to be manifested.

### **Mating success, gestation length and litter size**

100% mating success due to *Asparagus racemosus* is probably due to increased concentration of estradiol that promotes follicle maturation. It also causes pre-ovulatory gonadotropins surge leading to ovulation (Smith et al., 2005). The normal gestation length of 21 days probably was due to presence of estrogenic compounds in the *Asparagus racemosus* that may mimic the normal end of gestation high estrogen levels compared to ibuprofen that had slight increased number of days. Increased litter size due to effect of *Asparagus racemosus* extract is probably due to its reported enhancement of folliculogenesis and ovulation in Wistar rats (Kalia et al., 2003). *Asparagus racemosus* is also reported to play a role in uterine receptivity and implantation hence enhancing fertility (Prasad et al., 2002). Kumar and Singh, (2001) reported improved conception rate of due to *Asparagus racemosus* in women under-going IVF compared to the placebo. The 100% mating success caused by ibuprofen with prolonged gestation length and only two out of five rats littering and decreased litter size probably was due to compromised fertilization and/or implantation.

### **Myometrial quiescence**

The possible cause for dysmenorrhea is increased release of prostaglandins (PG) especially PGF2 $\alpha$  which cause hyper contractility of the uterine muscle in turn reducing uterine blood flow hence causing pain (Wallace et al., 2010). In this study, higher concentration of the extract caused the most significant reduction in the force and frequency of uterine contraction ranging from  $P < 0.01^*$ ;  $P < 0.001^{**}$  to  $P < 0.0001^{***}$  (Figure 2 and 3). These

results corroborates other studies (Khulbe, 2015; Mishra et al., 2013) that reported similar results. *Shatavari* blocked uterine contractions in the rat, guinea pig and rabbit in a dose dependent manner, hence used in the management of dysmenorrhea. *Echinophora platyloba* is reported to reduce uterine contractions hence its use in treatment of dysmenorrhea (Bahmani et al., 2015). *Achillea willhemsii* causes anti-prostaglandin effect hence used to manage dysmenorrhea (Mirabi et al., 2014). *Saraca indica* treats dysmenorrhea (Mishra et al., 2013). *Zingiber officinale* treats dysmenorrhea and menstrual irregularities (Ozgoli et al., 2009). *Foeniculum vulgare* inhibit uterine contractions induced by prostaglandins there-by minimizing dysmenorrhea (Shams et al., 2005). *Matricaria chamomilla* has antispasmodic effects thus used in the treatment of dysmenorrhea (Barene et al., 2003). Peppermint oil provides antispasmodic activity on the uterine muscle by blocking the calcium channels thus used in dysmenorrhea management (Bahmani et al., 2015). Plants have the potential to generate novel dysmenorrhea drugs and so does *Asparagus racemosus*. It is therefore imperative to pursue alternative source of remedies for dysmenorrhea. Saponins, glycosides and flavonoids in the *Asparagus racemosus* aqueous root extract might be responsible for the fertility effect and its use in pain relief. Effect of *Asparagus racemosus* on these reproductive parameters shows no adverse effects and can be used as fertility promoter. However further work can be done involving testing the plant on other smooth muscles. Also isolation of pure compounds so as to establish if the activity is attributed to specific compounds.

### **CONFLICT OF INTEREST**

We declare there was no conflict of interest.

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