

Research Article



Jussiaea repens (L) Acts as an Uterotonic Agent - An *In vitro* Study

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ABSTRACT

The ethno-biological concept about the use of herbal extract for parturition, lactation and sometimes for abortion is reported since ancient ages. Among such medicinal herbs, *Jussiaea repens* (L), commonly known as 'Keshardam' in Bengal, is a water creeping primrose, found in wet lands of Bengal and in other countries of the world also, has reported to have many medicinal uses in different fields i.e. antidiabetic, anti helminthic, anti inflammatory, antibacterial etc. Recently we have reported the plant as a nontoxic antigonadal herb in male rats. But there is no report yet regarding the effect of this plant extract in female reproductive system especially on uterus except in Papua New Guinea, where only ethno biological application of this plant has been reported to prevent pregnancy. So, the present study has undertaken to investigate whether the crude aqueous extract of *J. repens* (except root) has any effect on uterine contraction in *in-vitro* condition and also to study whether it's action can be compared with the action of oxytocin. Results of our pilot studies show that the crude aqueous extract of *Jussiaea repens* at a dose of 40mg dry extract / 30 ml physiological fluid in a bath, on isolated non pregnant uterus of adult female rats *in-vitro* causes significant increase of force and frequency of contraction than normal. The results (as percentage) have compared with the effect of oxytocin in presence of atropine (oxytocin blocker). The results show that the extract may act as oxytocin which is antagonized by atropine. So, from the above study it may be concluded that, the extract of *Jussiaea repens* acts as herbal uterotonic agent and so can be applied after full term pregnancy, which may favour the smooth parturition process like oxytocin.

Keywords: *Jussiaea repens* (L), Keshardam, Oxytocin, Oxytocic, Uterine contraction, Uterotonic.

INTRODUCTION

Herbal plants have been used as domestic medicine for child birth for decades and are well recognized in the field of Ayurveda from the ancient ages, which are now turned as medicinal plants.¹ Nowadays researchers are trying to develop different drugs with therapeutic uses from plant extracts. Researchers find that medicinal plant and their individual constituents act in similar fashion as the modern drugs and sometime better without the dreaded side effects.²⁻⁵

In the field of paediatrics and gynaecology, the complication in child birth and regulation of menses are the major problems in the present life style and culture. For amelioration, women are using synthetic drugs, which are costly and have so many side effects.⁶⁻⁸ So, in the modern trend, women are looking for safe herbal medicines for safe child birth and regulation of menses. So, researchers are interested in developing herbal medicine which are relatively cheap and have little or no side effect compared to synthetic one. Work is going on a large scale in different laboratories and in different pharmaceuticals for the development of herbal drugs at different formulations and preparations for different treatments.⁹⁻¹²

In our present study, we have selected a common Indian herb, locally known as Keshardam, scientifically called *Jussiaea repens* (L) of Onagraceae family¹³ which is a creeping water primrose, grows in fresh water, ponds, canals of road side and wetlands. Pharmacologists

reported the clinical uses of this plant as hepatoprotective, anti inflammatory, antidiabetic⁹ antibacterial¹⁰ and fibrinolytic agent.¹⁴ It is also reported that, in different Districts of West Bengal, Jharkhand, Orissa¹⁵ and Manipur¹⁶ in India, some tribal people (Oraon and Chero) unknowingly consume this plant as vegetable and animal forage.¹⁷ In Papua, New Guinea, the leaves and stem of this plant are considered to have contraceptive (preventing pregnancy) properties.¹⁸ Different authors reported that aerial parts of this plant is composed of different metabolites like, rutin, kaempferol, Quercetin, terpenes, triterpenes etc.^{10,19-21} Again, purified rutin, kaempferol, quercetin and triterpenes are potent antifertile compounds as reported by different investigators.²²⁻²⁵

Recently, we have reported the nontoxic antigonadal activity of this plant in a dose dependent manner^{2,26} and its action is mediated through alteration of different biochemical parameters of male reproductive system in rats.³ But no report yet is available, regarding the effect of this plant on female reproductive system. So, in this preliminary study, our aim is to study the effect of aqueous crude extract of *J. repens* (except root) on adult virgin rat uterus in *in-vitro* and also to study the nature and mode of action of the plant on isolated uterus whether it may act as abortifacient and or as oxytocic agent modulating uterotonic activity.



MATERIALS AND METHODS

Plant Material

The plant *Jussiaea repens* L. was collected from wetlands of 24 Parganas (N), WB, India, during the month of March - April. The material was identified and authenticated by taxonomist of Central National Herbarium (Kolkata), Botanical Survey of India (BSI), Shibpur, Howrah having voucher specimen number NP-01 dated 25.03.2011. The voucher specimen was deposited in the Botanical Survey of India (BSI) for future reference. Fresh plants were carefully washed under running tap water and finally with distilled water and air dried at 35-40°C for 4-5 days, then homogenized to a fine powder by mixer grinder and stored for extraction.

Extract Preparation

400 gm dry powder of *J.repens* was extracted in 4 L distilled water at 50°C for 30 minutes and allowed to cool and kept overnight at room temperature.²⁸ The residue was further extracted twice similarly with boiling water. The extract was filtered using a clean muslin cloth, ordinary filter paper and Whatman No. 1 filter paper accordingly. The resulting filtrate was then concentrated using a rotary evaporator and further dried at 40°C. Finally yield 6.25% solid crude extract was stored in powdered form in an air tight container at 4°C for further use in the experiment. The percentage extract yield was estimated according to Parekh and Chanda,²⁸ as: Dry weight/Dry material weight × 100.

Animal selection, maintenance and treatment

Six adult virgin female rats (white albino, *Rattus norvegicus* L. of Wistar strain) weighing 130g ±10 were selected for the experiment. The animals were acclimatized to the laboratory condition for a period of one week before starting the experiment. The animals were maintained under standard laboratory conditions 12 hrs. Light: 12 hrs. dark, temp. 25±2°C and relative humidity (40-60%) with free access to standard normal diet, prescribed by ICMR, NIN, Hyderabad, India,²⁹ and water *ad libitum*.

Animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) guided by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Ref.no. PU 796/03/ac/CPSEA. All efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were pretreated with diethylstilbestrol (Merk, Germany) at the dose of 1mg/kg body weight (i.p), 24 hrs before experiment³⁰ to make the uterus more sensitive to uterotonic agent, i.e. oxytocin.³¹

Isolation of rat uteri and contractility measurements in vitro

The animals were confirmed of oestrous stage by vaginal smear study³² and killed by cervical dislocation and exanginations.³³ After laparotomy, a piece of uterus (1.5cm) from both horns was excised and one was mounted in an organ bath containing 30 ml Tyrode's solution for recording of contraction rate (incubated at 37°C prior to tissue mounting, P^H 7.4) and another in De Jalon's solution (at 28°C, P^H 7.4) between two hooks bubbled with normal air.³³ The lower hook was fixed at the bottom of organ bath and the upper hook was connected to an end of isotonic and linear lever of Dale's apparatus (modified after Rosenberg, 1973).³⁴ The equilibrium period (no normal contraction) was 60 min, in which the bath solution was changed at every 15 mins.³⁵ Under 0.5g resting tension, a stylus recording system was used to record the uterine contractions on a smoked glossy paper in kymograph (E8 Kymograph, INCO Instruments and chemicals, Ambala, Punjab, India) drum, moved at 2.5mm/sec speed (the method was modified instead of polygraph after Mitra et. al 1999),³⁶ which was varnished after and calculated the data. The uterine response was recorded as -

Contraction rate

Normal contraction/min without any treatment, crude aqueous extract of *Jussiaea repens* (at the dose, 1ml containing 40mg dry extract in 30 ml physiological fluid in Dale's bath), oxytocin (0.001U/ml)³³ and atropine sulphate at the dose of 1mg/ml of water.³³

Force of contraction (in respect to height in mm)

The response of contraction force was calculated in respect to the % response of oxytocin (considering 100). The oxytocic nature of the extract was tested by infusion of atropine as the antagonist of oxytocin. After recording the effect of drug each time, the tissue was washed at least four times after each fifteen minutes interval.

The composition of commonly used Tyrode's solution/L was - NaCl (137 mM), KCl (2.68 mM), CaCl₂ (1.8 mM), NaHCO₃ (11.9 mM), MgCl₂ (1.05 mM), NaH₂PO₄ (0.42 mM) and glucose (5.55 mM), P^H 7.4. The De Jalon's solution was used for assay experiments with the following composition/L - NaCl (154mM), KCl (5.63mM), CaCl₂ 2H₂O (0.65mM), NaHCO₃ (5.95mM) and D-glucose (2.77mM), P^H 7.4.³⁷ Tyrode's solution was used for recording the normal contraction, where, because of two reasons De Jalon solution was used in oxytocin experiments, as the uterus contraction induced by oxytocin in the De Jalon solution was more stable than in Tyrode solution and also to prevent superimposing the effect of extract on the rhythmic contractions induced by oxytocin.³⁸ The salts and diethylstilbestrol were purchased from Merck (Germany), oxytocin and atropine from Sigma-Aldrich.



Statistical analysis

All the recorded values were expressed in mean \pm SEM. The data were analysed statistically by Student's t-test using SPSS version 16 software. The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Study of uterine contraction rate

The normal uterine contraction rate was counted per minute followed by infusion of single dose of oxytocin (0.001 U/ml of water), crude extract (1ml aqueous extract containing 40 mg powder) and atropine (1mg/ml of water). The contraction rate in presence of oxytocin and extract treated group was increased significantly and the rate was again significantly reduced in presence of atropine (oxytocin blocker) (Table 1, Figure 1,3).

Study of uterine force of contraction

The force of contraction of control was considered by administering distilled water³⁹ and was recorded as '0' (equal to normal). The height of contraction (in mm) in oxytocin treatment was considered as 100 or 100%. In respect to that, the contractions in extract and atropine treatment were calculated as percent.

The results in presence of oxytocin and plant extract on isolated uterus, were absolutely stimulatory. The contractile nature of uterus in presence of extract was mostly similar to that of oxytocin. The application of atropine (antagonist of oxytocin) at the dose of 1mg/ml of water on the normal uterine contraction caused spasmolysis (Table 2, Figures 2,3), which was also followed by significant inhibition in oxytocin and *J.repens* extract application following just after the application of atropine (Table 2, Figures 2, 3). Atropine, the antagonist of oxytocin, when administered simultaneously before oxytocin and extract, showed similar pattern of inhibition in compare to oxytocin and extract treatment alone (Table 2, Figure 3) in uterine contraction.

Table 1: Uterine contraction /min *in-vitro* of different groups in isolated preparation of uterus (n = 6, Mean \pm SE, ** $P < 0.01$, when compared with a/b, a/c, a/d).

Treatment	No. of contraction/min
Control (no treatment, only dil. water)	12.00 \pm 0.258
Extract – 1ml containing 40 mg powder	15.16 \pm 0.307**
Oxytocin (0.001 U/ml)	18.33 \pm 0.421**
Atropine (1mg/ml)	8.00 \pm 0.258**

DISCUSSION

The rat uterine tissue was used for ethanopharmacological screening of *Jussiaea repens* (L) as uterus has a unique property of auto rhythmicity and shows movement even in isolated condition. The smooth muscle of rat uterus was the point of interest for herbal

drug experimentation due to its high sensitivity among other laboratory animals. The aqueous extracts of *J. repens* at the dose of 40 mg/30 ml fluid, caused rise of both force and rate of contraction and increased the uterine motility alike the oxytocin (Table 1,2; Figures 1-3). This result supported the other finding with the aqueous herbal extract of *V. amygdalina* as a strong oxytocic plant. This herbal extract was found to be long acting and when introduced to the rat uterus, it caused contractions that were sustained over 30 minutes.⁴⁰ According to our findings; the plant extract was found oxytocic and might cause abortion when used in preterm pregnancy.

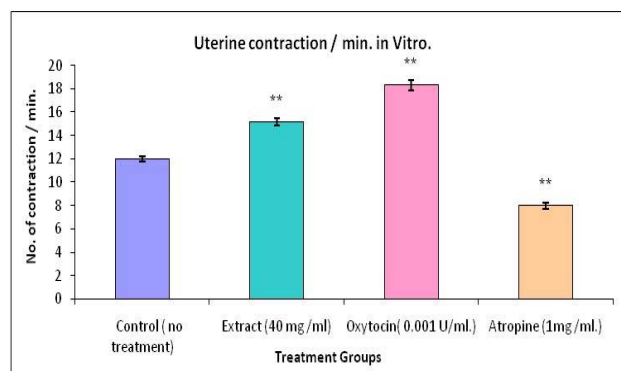


Figure 1: Uterine contraction rate (per min) *in-vitro* of different groups in isolated preparation of uterus. (n = 6, Mean \pm SE, ** $P < 0.01$, when compared with control).

Table 2: Uterine contraction force (height in mm, considered 100 % in oxytocin) *in-vitro* of different groups in isolated preparation of uterus (n = 6, Mean \pm SE, ** $P < 0.01$, when compared with b/c, b/d, c/e).

Treatment	Force of contraction (%)
Control (treatment with dil. water)	0.00 \pm 0.00
Oxytocin (0.001 U/ml)	100 \pm 0.00
Extract (1ml containing 40 mg powder)	78.33 \pm 0.210**
Atropine (1mg/ml) + Oxytocin (0.001 U/ml)	46.50 \pm 0.341**
Atropine (1mg/ml) + Extract (40 mg/ml)	54.50 \pm 0.957**

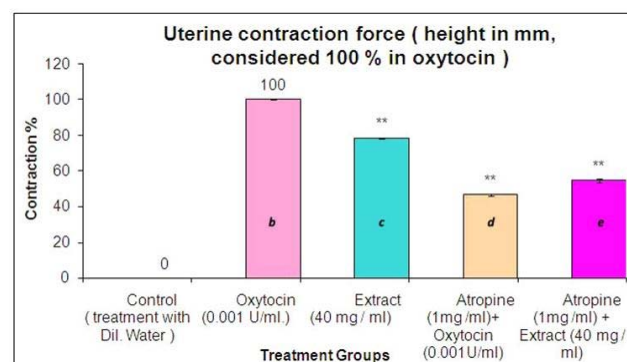


Figure 2: Uterine contraction force (height in mm, considered 100% in oxytocin) *in-vitro* of different groups in isolated preparation of uterus (n = 6, Mean \pm SE, ** $P < 0.01$, when compared with b/c, b/d, c/e).



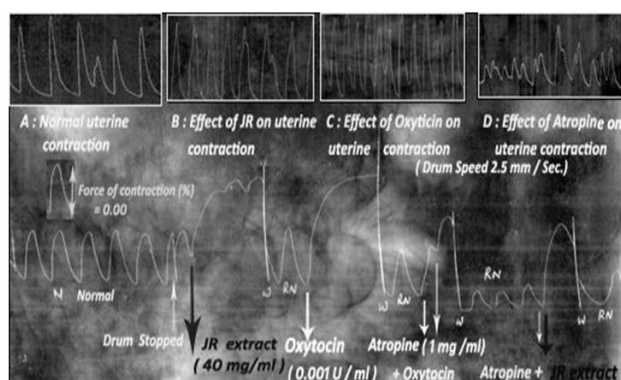


Figure 3: Kymographic tracing of composite study of uterine contraction with successive application of JR extract (40 mg /ml), oxytocin (0.001 U /ml), oxytocin followed by atropine (1 mg / ml) and JR extract followed by atropine *in vitro*. (N: Normal, W: Wash, RN: Recovery normal, **A:** Normal uterine contraction, **B:** Effect of JR on uterine contraction, **C:** Effect of oxytocin on uterine contraction, **D:** Effect of atropine on uterine contraction).

The usage of *J. repens* can also induce early labour, if due care is not taken and can cause uterine rupture or other complications to the mother and foetus but in full term pregnancy it may help in smooth parturition process. This uterine contractility has also other indications, that *J. repens* can hasten childbirth and lactating women may also consume as decoction to increase milk flow, as supported by other study.⁴¹ This primary work may also be explained through other studies i.e. in Guinea-Bissau and Nigeria, the infusion of leaves of *V. amygdalina* is used as an abortifacient in women.^{42,43} Again in Rwanda, the methanolic extract of *V. amygdalina* showed weak relaxant activity on the smooth muscle of guineapig ileum, but caused neither relaxation nor stimulation of the guineapig uterine muscle.⁴⁴ Although the ethnopharmacological experiments elsewhere never proved more effective, it could have been due to the choice of the test animals used since the rat uterus is more sensitive. In Uganda, *Cleome gynandra* is used to hasten childbirth, might be by augmenting endogenous Oxytocin concentration, through sustaining the force and amplitude of uterine contraction.^{37, 45} This also supports our present study and the mode of action of *J. repens*, which is similar to oxytocic plants like *V. amygdalina* which have the ability of hastening the parturition process.⁴⁰ The exogenous oxytocin or oxytocic substances can initiate or enhance rhythmic uterine contractions. Such uterine stimulating agents are used most frequently to induce or augment labour in selectively pregnant woman in hospitals, But the plant extract used by traditional birth attendants to hasten childbirth is limited to a common practice.⁴⁶ So, in this study, the *J. repens* plant extract has oxytocic nature, which has been confirmed further by administrating atropine as an oxytocin antagonist. The percentage inhibition by atropine on direct effect of oxytocin and the plant extract, show the oxytocic as well as utero tropic action of the plant extract, which is further supported by another

experiment³³ following administration of dual antagonist like atropine and salbutamol against oxytocin and the herbal drug parallel.

CONCLUSION

The aqueous extract of *J. repens* (except root) has uterotonic effects on uterine contraction in rats. So, this study could justify the use of the extract of *J. repens* in traditional medicine to facilitate childbirth, milk ejection, placental expulsion, abortion etc. Further study is required to establish and confirm the truth and to make it a patent herbal medicine.

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