ABSTRACT

Objective: Nymphaea alba is a medicinal plant used in tropical regions for its genital, gastrointestinal and bronchial activities. In the present study, the probable effect of the ethanol extract of the rhizome on rat uterus in the presence of standard antagonists like salbutamol and atropine and a uterine stimulant like oxytocin were examined.

Methods: This work examined the effect of the ethanol extract of the N. alba rhizome on rat uterus pre-treated with 1 mg/kg stilboesterol for 24 h. The effects of oxytocin, a uterine contraction agonist, antagonists like atropine (1-2 mg) and salbutamol (2 μg) on the uterine contractile effect of the extract were investigated.

Results: The ethanol extract of N. alba produced a dose related increase in the force of uterine contraction similar to Oxytocin. The drug Oxytocin was observed to potentiate the uterine contractile activity of the extract while pre-treating the tissue with either atropine or salbutamol before administering the extract showed the inhibitory effects of the drugs on the activity of the extract.

Conclusion: The inhibition of contractile effect of the N. alba extract showed by atropine and salbutamol suggests the probable stimulation of the muscarinic and adrenergic receptors of the uterus by the extract. These findings justify the traditional use of the plant for its uterotonic properties.

Keywords: Nymphaea alba, Uterine, Oxytocin, Atropine, Salbutamol, Prostaglandin.

INTRODUCTION

Most of the plants claimed to be oxytocic used to induce and maintain labour, aid the removal of retained placenta, regulate postpartum bleeding and act as abortifacient [1]. Plants that produce uterine contractions have a similar action to that of oxytocin which stimulates the uterus, causing strong contractions, and thus producing labour [2, 3]. However, if used during the first months of pregnancy, they could have abortifacient properties [4].

Traditional birth attendants, mothers-in-law, mothers and the expectant mother mostly prescribe these herbal remedies to induce labour. Some of these medicinal plants are also fed to cows and goats in labour. The persistent use of plants by pregnant women and traditional birth attendants for the induction of labor suggests that some herbs might be potent uterine stimulants. Therefore, studies of such herbs could provide a helpful guide to the discovery of new oxytocs.

Nymphaea alba, syn. Nymphaea rubra, white water lily (Nymphaeaceae) occurs in temperate and tropical regions. There are approximately 50 species in this genus. Most are aquatic herbs, which are widely distributed in India, from Bengal, Orissa to Kashmir in lakes and ponds. The roots have also been used extensively in a variety of folk medicine. The dried root and rhizome of the white water lily have been used orally to treat gastrointestinal, genital, and bronchial conditions [5]. The leaves and roots have also been used externally, as infusions to treat lesions and inflammation associated with mucous membranes, and as poultices to treat a variety of dermatological conditions [6]. A tea often made from the plant that is used to combat kidney and bladder problems [7]. The mucilaginous and somewhat acrid root and stock are administered in some countries for dysentery. It is an astringent and slightly narcotic medicine [8]. The flowers are reputed to be anti-aphrodisiac. An infusion of the flower and fruit is given in diarrhea and as a diaphoretic [9]. But scientific investigations on its effect in the reproductive system has not yet been performed which may support its use as traditional use. Thus, in the present study, the probable effect of the ethanol extract of the Nymphaea alba rhizome on rat uterus in the presence of standard antagonists like salbutamol and atropine and a uterine stimulant like oxytocin were examined.

MATERIALS AND METHODS

Plant material

The various parts of several young mature plants (root, rhizome) were collected in bulk from Salipur, Cuttack district, Orissa, India, in September 2009 and authenticated by a taxonomist. A voucher specimen has been retained in our laboratory for future reference.

Preparation of extracts

The rhizome were shade dried and pulverized using an electric grinder and was extracted by taking 200 grams of the powder and soaked in 2000 ml of 90% ethanol for 3 days. The mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The resultant filtrate was evaporated to dryness in a steam bath to give a yield of 8.0% (w/w) of the extract.

Animals

Virgin female wistar rats (120-130 g) obtained from Chakraborty Animal Centre, Kolkata, India was used. The animals were kept in a well-ventilated room in the Laboratory Animal Centre of Institute of Pharmacy and Technology, Orissa. They received standard food and water ad libitum. The study was undertaken with due approval of the study protocol by the Institution Animal Ethics Committee (1053/ac/07/CPCSEA) and the experiments were performed according to the current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Drugs and chemicals

Atropine (Indus Pharma, New Delhi, India), salbutamol, stilboesterol, oxytocin (G. Richter, Vapi, India), D-glucose, potassium hydrogen phosphate, magnesium sulphate heptahydrate, calcium chloride dihydrate (Merck, Mumbai, India), sodium chloride, sodium hydrogen carbonate (BDH Chemicals, Mumbai, India), and potassium chloride (Cambian Chemicals, Alwar, India) were used in the experiments.

Isolated organ preparation

To obtain the estrogenized uterus, virgin female rats were subcutaneously injected with 1 mg/kg stilboesterol 24 h before the
experiment. The rats were sacrificed by a blow on the head followed by exsanguination. After opening the peritoneal cavity, approximately 1.5 cm of the uterine horns were removed and cleaned free from fatty and connective tissues. Each uterine strip was suspended in 50-ml organ baths containing De Jalon’s physiological solution (composed of, in g/l: NaCl, 9.0; NaHCO₃, 0.5; glucose, 0.5; KCl, 0.42; MgCl₂, 0.006; CaCl₂·2H₂O, 0.08) maintained at 36±1°C and aerated with 5% CO₂ in O₂. [Jaybhaye et al. 2010]. The uterine strips were connected to Ugo Basile isometric force displacement transducer connected to Ugo Basile (7050) uni recorder (Comerio VA, Italy) which measured the mechanic responses. The transducer was previously calibrated to establish a relationship between the force applied to the transducer and gauge deflection with a 500 mg corresponding weight. The preparations were allowed to equilibrate for at least 30 min before the administration of the extract or drugs.

Drug challenges

After 30 min of equilibration period, uterine contractile responses were elicited by adding non-cumulatively oxytocin at doses 0.02-0.16 i.u/ml (1 mg of synthetic oxytocin corresponds to 500 i.u.) and ethanol extracts of *N. alba* (25-200 mg/ml) to the De Jalon solution. Each dose of the drugs was allowed to act for 10 min and the amplitude of contraction recorded. The contractions were recorded by means of an isotonic transducer (Ugo Basile, Italy) connected to a single channel recorder (Ugo Basile) which was calibrated to record change in the tension generated on g versus cm displacement basis. The tension applied to the preparation was 0.71 g. Atropine (2 mg) and salbutamol (2μg) were used to antagonize in a concentration-dependent manner the maximal response of the isolated uterus to oxytocin (0.02-0.16 i.u/ml) and extracts of *N. alba* (25-200 mg/ml) 10 min before addition of plant extract or standard agonists as oxytocin.

Data analysis

Data presented are mean ± standard error (S.E.) for three replicates. The uterine contractile readings obtained for *N. alba*, oxytocin and their combinations were compared to control readings (i.e., spontaneous uterine contraction in absence of any drug) using student’s t-test (with significance at P< 0.01). Whereas the antagonistic effect of Atropine and salbutamol were evaluated with the corresponding readings of *N. alba* alone by paired t-test (with significance at P< 0.01).

RESULTS

Both oxytocin and the ethanol extract of *N. alba* rhizome induced a dose-related increase in force of contraction of the isolated rat uterus (fig.1). Addition of varying concentrations of oxytocin to the tissue elicited a dose dependent uterine contraction. While 0.02 i.u produced a force of contraction of 1.3 ±0.22 g, maximum contraction was obtained with the administration of 0.16 i.u which produced a force of 1.88 ±0.08 g. While 25 mg of the extract elicited a mean force of contraction of 1.12 ±0.15 g, administration of 200 mg produced a corresponding maximum uterine force of contraction equivalent to 1.68 ±0.6 g. Hence, the uterine contractile effect produced by 200 mg of the extract was observed to be very similar in magnitude to that produced by 0.16 i.u. oxytocin. Moreover, simultaneous administration of oxytocin and the extract produced uterine force of contractions significantly higher than either oxytocin or extract alone (fig.2).

![Fig. 1: Comparison of various doses of oxytocin and *N. alba* extract on uterine contractions. Values are expressed as mean ± S.E (N=6). *P<0.01.](image)

Administration of atropine did not evoke any effect on the activity of the uterus but significantly inhibited the contractile activity of the extract in a competitive and dose-dependent manner. Pre-treating the tissue with 2 mg atropine decreased the uterine contraction of 1.54 ±0.25 g elicited by the 100 mg of the extract to 0.2 ±0.01 g. Administration of 2 mg of atropine completely inhibited the contraction induced by the 25 mg concentration of the extract. Contractions induced by 200 mg of the extract were also significantly inhibited by 2 mg atropine (P< 0.01) (fig.3).

![Fig. 2: Effect of simultaneous administration of oxytocin and *N. alba* extract. Values are expressed as mean ± S.E (N=6). *P<0.01.](image)

![Fig. 3: Inhibitory effect of atropine on uterine contraction of various doses of *N. alba* extract. Values are expressed as mean ± S.E (N=6). *P<0.01.](image)

Salbutamol was observed to show remarkable inhibition of uterine contraction elicited by the ethanol extract. Administration of 2 μg salbutamol before 25 and 100 mg/ml of the ethanol extract
Whereas the contraction exhibited by oxytocin though not mg/ml showed a progressive decrease in observed contraction up to 20 mg/ml, increase in concentration after this, that is, > 20 progressive increase in contraction at initial low concentration further increase in magnitude. The progressive decrease at i.u/ml after which increase in concentration did not produce after 0.1 i.u/ml with progressive increase in magnitude to 1.0 strong at the lower concentration of < 0.1 i.u/ml was stronger the structure of the active ingredients.

increase in contraction of the isolated rat uterus. The extract, produced a concentration-dependent increase in contraction of the isolated rat uterus. Concentration-dependent inhibitory effects of atropine and salbutamol on the maximum contractile response of the isolated estrogenized uterus to the ethanol extract of N. alba (25-200 mg) were also observed.

Oxytocin is a mammalian neurohypophysial hormone that acts primarily as a neuromodulator in the brain. It is on the World Health Organization’s List of Essential Medicines, a list of the most important medication needed in a basic health system [10]. Oxytocin plays an important role in sexual reproduction, in particular during and after childbirth. It is released in large amounts after distension of the cervix and uterus during labor, facilitating birth, maternal bonding and, after stimulation of the nipples, lactation. [11]. Oxytocin is also used in veterinary medicine to facilitate birth and to stimulate milk release. Injected oxytocin analogues are used for labor induction and to support labor in case of difficult parturition. However currently used intravenous therapies to induce uterine contractions lack potency/selectivity and can have harmful side effects for mother and child. A great number of medicinal plants are also known for their oxytocic potentials [12-15].

The oxytocic screening of ethanolic extract of Nymphaea alba exhibited agonistic effect which are comparable in magnitude with oxytocin. This observed uterus-contracting action of ethanolic extract of Nymphaea alba was fast in onset and could be totally eliminated by washing with the extract free De-jalon solution. This may suggest the presence of low molecular weight active compound(s) in the extract, which penetrated rapidly to its site of action. While ethanolic extract exhibited a strong and progressive increase in contraction at initial low concentration up to 20 mg/ml, increase in concentration after this, that is, > 20 mg/ml showed a progressive decrease in observed contraction. Whereas the contraction exhibited by oxytocin though not strong at the lower concentration of < 0.1 i.u/ml was stronger after 0.1 i.u/ml with progressive increase in magnitude to 1.0 i.u/ml after which increase in concentration did not produce further increase in magnitude. The progressive decrease at higher concentration with time in the oxytocic action of ethanolic extract of N. alba may be due to metabolic changes in the structure of the active ingredients.

Many studies have indicated the existence of abundant cholinergic receptors in the uterine smooth muscle and that stimulate uterine contraction. The effect of the plant extract was evaluated in the presence of adequate antagonists of these substances. Thus, pre-treatment of uterine strips with atropine (2mg/ml) and salbutamol (2µg/ml) antagonized concentration dependently the maximal response to the plant extract (25-200 mg/ml). These results imply that the bioactive compounds found in the ethanolic extract of Nalba appear to activate the endometrial and myometrial cell membrane receptors resulting in an uterine effect by a mode of action possibly via the prostaglandin synthesis and the activation of cholinergic, oxytocic and adrenergic receptors.

In order to ascertain the involvement of cholinergic and adrenergic pathways in the mechanism of N. alba induced uterine contractions, the effect of the plant extract was evaluated in the presence of adequate antagonists of these substances. Thus, pre-treatment of uterine strips with atropine (2mg/ml) and salbutamol (2µg/ml) antagonized concentration dependently the maximal response to the plant extract (25-200 mg/ml). These results imply that the bioactive compounds found in the ethanolic extract of N. alba may account for the observed uterine contractile activity. Moreover, the ethanolic extract of N. alba has already reported to be well tolerated in acute toxicity studies by the authors [30]. These findings justify the traditional use of the plant for its uterotonic properties. Further studies are needed to clearly elucidate the mode of contraceptive action of this medicinal plant.

**REFERENCES**

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