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## Uterotonic activity of *Heimia salicifolia* leaves

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#### Abstract

This paper aimed to test whether the extracts of *Heimia salicifolia* leaves contract isolated uterus of estrogenized rats. *H. salicifolia* (Lythraceae) is a shrub used in traditional medicine as abortifacient in Mexico. Leaves of *H. salicifolia* were extracted with water, methanol and chloroform. Uterotonic activity was studied in the isolated uterus of Wistar female rats, previously oestrogenized with diethylstilbestrol (1 mg/kg, sc). After 24 h the uterus was obtained and placed in an isolated organ chamber with constant bubbling of a mixture of O<sub>2</sub>/CO<sub>2</sub> 95:5. 30 °C. Concentration-response curves were performed to oxytocin (Control), aqueous and methanolic extracts. Maximum response to oxytocin was observed with 0.8 ng/ml, to aqueous extract with 450 µg/ml and methanolic extract with 271.1 µg/ml. Chloroform extract (108 µg/ml) showed uterotonic effect and was inhibited with indomethacin. *Heimia salicifolia* showed oxytocic activity and explain their use in traditional medicine as abortifacient.

**Keywords:** *Heimia salicifolia*, uterotonic activity, oxytocin, oestrogenized rat uterus

#### Introduction

The mammalian uterus comprises an outer myometrium and an inner endometrium layer [1]. Uterine myometrial cells are responsible for contraction of the uterus whereas endometrial cells are secretory and non-contractile. The myometrium consists of circular and longitudinal muscles which differ in structure, function and contraction patterns. During parturition the myometrium contracts rhythmically and forcefully [2]. The contractions are induced by the secretion of oxytocin from the posterior pituitary gland. Oxytocin has clinically been used to initiate labor [3] as well as manage cases of post-parturition hemorrhage. The levels of oxytocin and oxytocin receptors in the myometrium have been found to be higher at term than at other periods [4] plays a crucial role in the expulsive stage of labor and the involution of the uterus [5]. The uterine contraction in turn stimulates increased secretion of oxytocin.

Uterotonic plants, are plants that stimulate uterine contraction and have been used since the ancient times to assist labor, remove the retained placenta, treat post partum bleeding and as an abortifacient [6]. Despite their use, scientific evidence to substantiate their beneficial effects are still being explored. Several plant extracts have been reported to induce uterine contraction which include the leaves extract of *Parquetina nigrescens* [7], *Caesalpinia bonduc* and *Agapanthus africanus* [9], seeds extract of *Carica papaya* [10] and *Labisia pumila* [11].

*Heimia salicifolia* (H.B & K.) Link & Otto (Lythraceae) is a wild flowering shrub distributed over Mexico, Western Texas, El Salvador, Jamaica, and South America (Uruguay to Argentina) [12-13]. The plant has different folk names viz. hauchinal, sinicuichi, in different places [12]. It has been in use in Central and South America as antisiphilitic, antipyretic, emetic, hemostatic, general tonic, laxative, diuretic, anti-inflammatory and for its wound healing activity [12].

The present study therefore examined the uterotonic activity of the aqueous, methanolic and chloroform extracts of leaves of *Heimia salicifolia* on the isolated uterus of oestrogenized rat.

#### Materials and Methods

##### Plant material and preparation of the extracts

Dried leaves of *H. salicifolia* were obtained from the Sonora Marketplace of Mexico City a marketplace specialized in selling medicinal plants. The identification was carried out by verified in the Herbarium of the Botany Department of the Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (UNAM). The specimen deposited with the voucher number 41653, was authenticated by Edith Lopez Villafranco, Biologist in charge

of the herbarium.

### Aqueous extract

The leaves were air-dried, cut into small pieces and grounded powder form. Each weighed powder parts (5 g) and boiled in 100 ml of distilled water. Then the aqueous extract was concentrated by heating at 40 °C and subjected to dry then stored in a container until use. Stock solution was obtained by dissolving small aliquots of the water extract.

### Methanolic extract and chloroform extract

200 g of dried leaves of *H. salicifolia* and 4 liters of double distilled water was added, it was boiled for 1 h and allowed to cool, then filtered and the aqueous extract was dried and pulverized. Proceeded to conduct extraction with methanol (1:20 p/v) at room temperature, it was concentrated and dried. The dried methanol extract was dissolved in water and was subjected to a chloroform extraction, which is concentrated and dried.

### Evaluation of pharmacological activity

Female Wistar rats (200-250 g body weight) were used and maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted in accordance with the *Federal Regulations for Animal Experimentation and Care* (NOM-062-ZOO-1999, SAGARPA, México), and were approved by the Institutional Bioethics and Investigation Committee.

Female Wistar rats were injected with diethylstilbestrol in arachis oil (1 mg/kg, i.p.) A day after injection, the rats were sacrificed and the uteri were immediately dissected out and opened longitudinally. The endometrium and most of the circular muscle layer were gently stripped away and the myometrial strips (15 mm long) and placed into a physiological solution. The tissue was then placed vertically in an organ bath containing solution with the following electrolytes composition in mM: 154 NaCl, 5.6 KCl, and 1.0 MgCl<sub>2</sub>, 0.54 CaCl<sub>2</sub> and 2.78 glucose while the pH was maintained at 7.4. The temperature of the organ bath was maintained at 37°C. 95% O<sub>2</sub> and 5% CO<sub>2</sub> was continuously delivered into the bathing solution. Each uterine strip was placed under optimum resting force of 1 g and was allowed equilibrate for 30 min prior to drug administration. During this period, the strips were washed with 10 ml fresh physiological solution of Jalon every 15 min according to the method by Oropeza *et al.*, 2013<sup>[14]</sup>. Each experiment was repeated six times using new uterine strips from different rats (n = 6). Contractile forces were recorded isometrically F-60 (Narco Bio Systems) by a force transducer which was connected to a bridge amplifier (DMP-4B, Narco Bio Systems)

The myometrial strips were allowed equilibrate for a period of about 30 min and then cumulative dose-response curves were constructed<sup>[15]</sup>. At 30 min intervals using oxytocin standard.

Administration of test drugs was only undertaken after equilibration and each subsequent test was preceded and followed by an oxytocin control curve. The tissues were preincubated for 5 min with the extracts and/or indomethacin and for 15 min. The tissues were allowed rest for at least 30 min between drug challenges and the organ baths were well rinsed during this period. The recovery period required for the myometrial preparation to respond normally to agonist stimulation was carefully monitored using matching uterine horns as time-matched controls and a time-matched control curve was constructed and used to monitor all test and internal

control curves.

### Drugs and chemicals

Oxytocin, indomethacin and potassium chloride salt (Sigma Aldrich, UK) were constituted in distilled water prior to use. To the aqueous extract of *H. salicifolia* curve was performed a dose - response at 20, 40, 60, 80 and 160 µg/ml.

### Study on oxytocin-induced uterine contractions

In other sets of experiments, uterine segments curve was performed a dose- response at 0.2, 0.4, 0.6, 0.8 y 1 nM of oxytocin, which from preliminary experiments was sustained over a 1 h period and as such allow proper evaluation of the plant extracts, fractions or standard drugs via cumulative additions every 10 min. The amplitude of contraction (maximum tension above basal force) and frequency of uterine contractions were obtained during the 10 min period following each sample addition where necessary.

### Statistical analysis

Results were expressed as mean ± SEM. Statistical comparisons were performed by one-way analysis of variance (ANOVA);  $p \leq 0.05$  was considered be statistically significant.

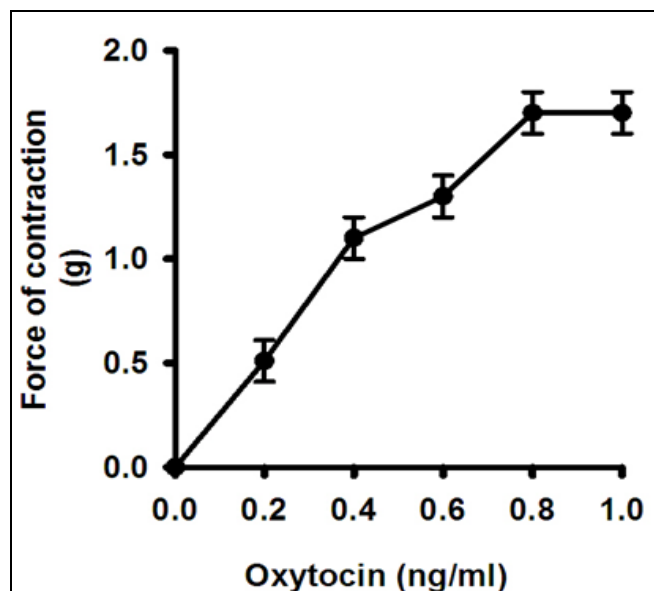
### Results

#### Performance of leaf extracts

From 200 g of dry leaves of *H. salicifolia* 34.64 g (17.32%) of the aqueous extract, 14.79 g (7.4%) of the methanol fraction and 1.0 g (0.5%) of the chloroform fraction was obtained.

#### Dose-dependent effect of oxytocin on uterine contraction

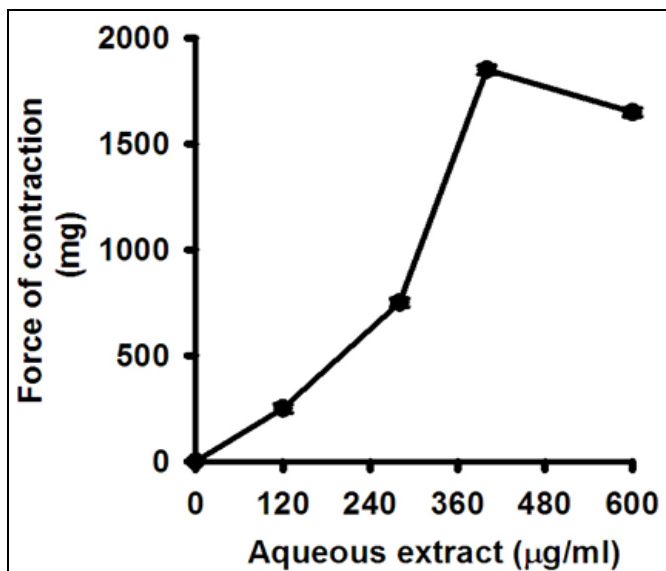
In Fig. 1, the force of contraction increases with increasing doses of oxytocin. In the control group, the force recorded was 1g tension, which was the baseline contraction in oestrogenized rats' uteri. Contractile force increases as the concentration of oxytocin,



**Fig 1:** Effect of oxytocin on uterine contraction. Mean tension generated from five isolated uterine horns obtained from different oestrogenized rats, which were exposed to various doses of oxytocin at concentrations ranging between 0.20 to 1 ng/ml. There was a dose-dependent increase in the tension with increasing doses of oxytocin. Values are the mean ± SEM; n = 5.

### Dose-dependent effect of aqueous extract of the leaves of *H. salicifolia* on uterine contraction

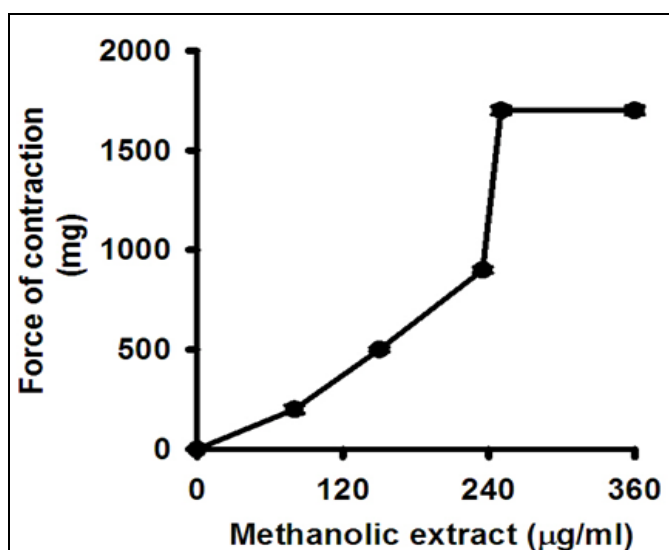
In Fig. 2, the force of contraction increases with increasing doses of aqueous extract of leaves of *H. salicifolia*. The force of contraction of the uterus dependently increased the concentration of the extract, with the maximum concentration of 450  $\mu\text{g/ml}$  the contractile effect was observed. These results show that *H. salicifolia* leaves contain oxytocic compounds.



**Fig 2:** Effect of the aqueous extract of leaves of *Heimia salicifolia* in preparation of isolated uterus of oestrogenized female rat. Values are the mean  $\pm$  SEM; n = 5.

### Dose-dependent effect of methanolic extract of the leaves of *Heimia salicifolia* on uterine contraction

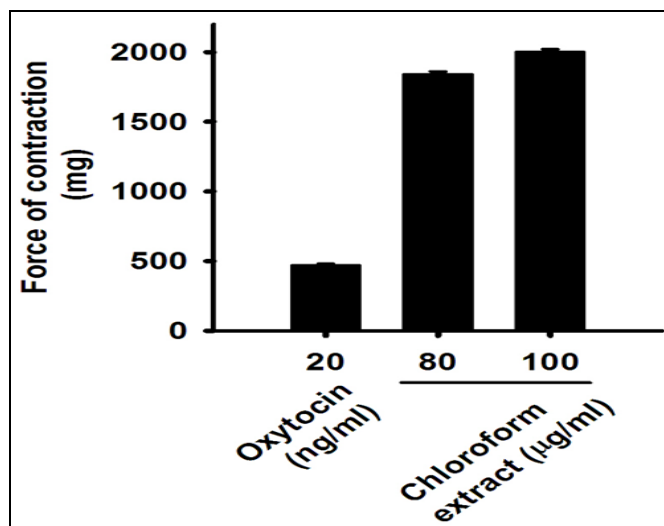
In Fig. 3, the force of contraction increases with increasing doses of methanolic extract of leaves of *H. salicifolia*. The force of contraction of the uterus dependently increased the concentration of the extract, with the minimum of 71.7  $\mu\text{g/ml}$  and maximum concentration of 271.1  $\mu\text{g/ml}$  the contractile effect was observed.



**Fig 3:** Effect of the methanolic extract of leaves of *H. salicifolia* in preparation of isolated uterus of oestrogenized rat. Values are the mean  $\pm$  SEM; n = 5.

In Fig. 4 is showed that chloroform extract of *H. salicifolia*

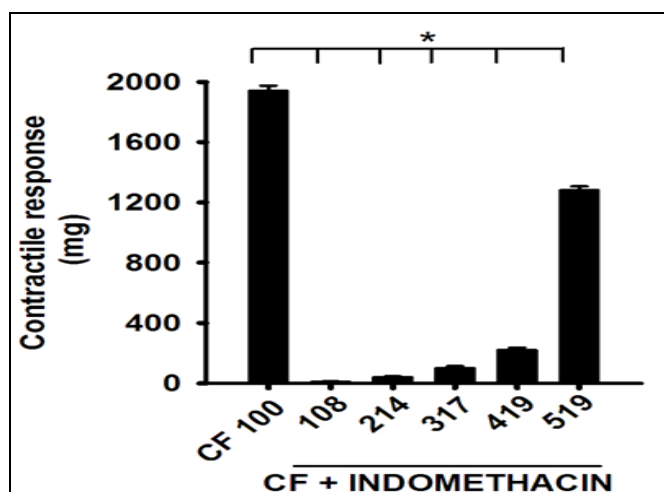
(80 and 100  $\mu\text{g/ml}$ ) and oxytocin (20 ng/ml) induce contractility of the isolated uterus of oestrogenized rat.



**Fig 4:** Effect of treatment with chloroform extract of *H. salicifolia* (100  $\mu\text{g/ml}$ ), oxytocin (0.2 and 0.8 ng/ml) on contractility of the isolated uterus of oestrogenized rat. Values represent the means  $\pm$  SEM of 5 rats per group.

### Inhibition of the uterotonic effect *H. salicifolia* in the presence of indomethacin

A fragment isolated uterus of oestrogenized rat was pretreated with indomethacin (100  $\mu\text{g/ml}$ ), a cyclooxygenase inhibitor, for 30 min. After the chloroform extract of *H. salicifolia* (108 to 519  $\mu\text{g/ml}$ ) was applied and uterus contraction was inhibited by indomethacin (Fig. 5). The inhibitory effect of indomethacin is partially overcome with the concentration of 519  $\mu\text{g/ml}$  of chloroform extract of *H. salicifolia*.



**Fig 5:** Inhibition of uterotonic effect of chloroform extract of *Heimia salicifolia* (CF) (108 to 519  $\mu\text{g/ml}$ ) in isolated oestrogenized rat uterus by pretreatment with indomethacin (100  $\mu\text{g/ml}$ ) for 30 min. Values are expressed as mean  $\pm$  S.E.M; n=5; \*p  $\leq$  0.05 versus chloroform fraction 100  $\mu\text{g/ml}$  (CF100).

### Discussion

The ethno pharmacological evaluation of a traditional herbal remedy must rely on an appropriate pharmacological model. The preparation of isolated uterus of oestrogenized rat is a model well suited to pharmacological investigations of contractile activity in the uterine smooth muscle and is used extensively by reproductive pharmacologists. Basic initial

pharmacological screening procedures which identify direct contractile activity at uterine smooth muscle of crude plant extracts; this study need to be followed by more detailed studies which characterize the pharmacological activity of the phytomedicine as fully as possible <sup>[16]</sup>.

#### **Aqueous, methanolic and chloroformic extracts of *Heimia salicifolia***

Leaves of *Heimia salicifolia* are used routinely for medicinal purposes <sup>[12]</sup>; however, their potency as uterotonic agents has not been documented. In the study by Kaingu *et al.* (2011) <sup>[17]</sup>, Traditional Birth Attendants administered the herbs extracts in the latter part of gestation, during labor and the immediate post-partum period, to ease the parturition process and to manage cases of post-partum hemorrhage and retained placenta after birth. The success of pregnancy depends on the ability of the myometrium to maintain quiescence throughout the duration of the gestation period. Premature onset of uterine contraction is often the cause of abortion. On the other hand, inadequate and infrequent contractions can result in delayed, obstructed or protracted labor and retained placenta after birth. The aim of the present study was to investigate the possible contractile effects of three extracts of leaves of *H. salicifolia* on uterine tissue. The results demonstrate that aqueous, methanolic and chloroform extracts of *H. salicifolia* caused increased contraction of isolated uterine tissue. Oxytocin is one of the most potent uterotonic agents known, and its effect on uterine contractility is of major pharmacological importance. Medicinal plants with oxytocic values that can be used to either induce labor or manage post-partum hemorrhage and retained placenta after birth and medicinal plants are of great importance especially in rural parts of developing countries where hospitals are not only far from rural homesteads but also have inadequate supplies of emergency medicine. Oxytocin is not only uterotonic by itself but also induces prostaglandin E<sub>2</sub> synthesis in uterine endometrial cells. This increase local prostaglandin production further stimulates uterine contraction.

Pretreatment by 30 min with indomethacin, inhibitor of cyclooxygenase activity, and posterior addition of chloroform extract showed inhibition of uterine contraction, suggesting that *H. salicifolia* extract induces uterotonic activity through prostaglandin synthesis <sup>[15, 18]</sup>.

Whole uterine tissue comprises an outer myometrium of longitudinal and smooth muscle cells, and inner endometrium <sup>[1]</sup>. Oxytocin causes uterine contractions by increasing [Ca<sup>2+</sup>] via two distinct mechanisms: calcium influx via the L-type Ca<sup>2+</sup> channels and calcium release from the sarcoplasmic reticulum. This pharmacological differentiation has been shown in both the rat uterus and pregnant human uterus <sup>[19]</sup>. The two hormones (oxytocin and prostaglandin) then synergistically affect myometrial contraction. Extracts of *H. salicifolia* stimulate uterine smooth muscle directly <sup>[20]</sup>.

#### **Conclusion**

Using in-vitro model, our study has provided the first scientific evidence to support the claim that *Heimia salicifolia* stimulates uterine contraction. The active compound that is responsible in mediating this effect is currently unknown, although *H. salicifolia* has been reported to contain alkaloids and flavonoids. This in-vitro study using isolated rodent's uteri therefore provides preliminary evidence which could be used to further explore the in-vivo effect of this plant compound on uterine contraction.

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