



## *In-vitro* Uterotonic activity of Involon

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### Abstract

Four successive extracts (petroleum ether, chloroform, methanol and water) of Involon(a polyherbal preparation of M/s Natural Remedies Pvt. Ltd., Bangalore) were tested for *in-vitro* uterotonic activity using isolated rat uterus. The activity was compared with commercially available synthetic Oxytocin and the methanolic extract of Involon was found to be most active among the extracts tested.

**Key words :** Involon, Uterotonic, Oxytocin, isolated rat uterus.

### 1. Objective

The present investigation was carried out to study the effect of four successive extracts (petroleum ether, chloroform, methanol and water) of Involon, a patented veterinary product of M/s Natural Remedies Pvt. Ltd., Bangalore for *in-vitro* uterotonic activity.

### 2. Test material

Involon, a polyherbal formulation contains hydroalcoholic extracts of *Peganum harmala* (seeds), *Adhatoda vasica* (leaves), *Gloriosa superba* (tubers), *Lepidium sativum* (seeds), *Gossypium herbaceum* and *Plumbago zeylanica* (roots). The product is meant for the expulsion of placenta after parturition in ruminants.

### 3. Preparation of extracts

Successive extracts (petroleum ether, chloroform, methanol and water) of Involon were prepared

using Soxhlet apparatus (yield : 0.4, 1.9, 36.1 and 22.66% w/w respectively on dry basis). The preliminary phytochemical screening gave positive test for alkaloids (chloroform and methanolic extracts) [1]. The extract residues were solubilized in water using Polysorbate 20 (2.5 %), Polyoxyethylene 40 Hydrogenated castor oil (1%) and Propylene glycol (3%) to obtain a final concentration of 2.5% w/w.

### 4. Tested activity

#### 4.1 *In-vitro* uterotonic activity.

This was done as per the method of Ghosh and Mary *et al.* [2-3]. In brief, female rat weighing 200 g was given diethyl stilbesterol in the dose of 0.2 mg/kg body weight subcutaneously, 36 – 48 h prior to the day of experiment in order to make the uterus more sensitive to uterotonic agent [4].

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On the day of experiment, the animal was anesthetized with pentobarbitone sodium in the dose of 35 mg/kg body weight. The abdominal wall was cut opened and uterus was identified as a fleshy bicornuate structure. Both the horns were cut near the vaginal end.

One of the horns of uterus (1-2 cm in length) was separated and transferred to a petri dish containing De Jalon's solution [5]. It was mounted in the organ bath maintained at 37°C. The tissue was connected to the force transducer and then to the Polyrite (Recorders and Medicare Systems, Chandigarh, India). The tissue was kept under 0.5-1 g resting tension and allowed to equilibrate for 30 min. The De Jalon's solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide.

Dose response curve was established with Oxytocin  $4 \times 10^{-5}$ ,  $2 \times 10^{-4}$ ,  $1 \times 10^{-3}$  and  $5 \times 10^{-3}$  mcg/ml of organ bath fluid to check the tissue sensitivity. Then all the solubilised extracts were tested at 75 mcg/ml of organ bath fluid. Together Polysorbate 20, Polyoxyethylene 40 Hydrogenated castor oil and Proplene glycol were tested and found free from any effect on uterus. Percent activity of extracts with respect to Oxytocin were calculated considering the contraction height of the Oxytocin  $5 \times 10^{-3}$  mcg/ml as 100%.

**5. Results :** As reported in Table-1.

Table 1.

Uterotonic activity of successive extracts of Involon

Test Material	Tested concentration (mcg/ml)	Percent contraction
Hydroalcoholic extract	75	33.91 ± 2.30
Petroleum ether extract	75	NIL
Chloroform extract	75	NIL
Methanol extract	75	74.14 ± 4.09
Water extract	75	20.60 ± 5.38
Oxytocin <sup>a</sup>	$5 \times 10^{-3}$	100

Values are in Mean ± SEM ; All determinations were done in triplicates; <sup>a</sup> Reference standard.

## 6. Conclusion

The present results suggests that the methanolic extract of Involon has a potent uterotonic effect, which is desirable for the expulsion of placenta in ruminants [6]. Since the activity is localised in two different extracts, it appears that there exists more than one uterotonic principle responsible for the uterotonic activity of Involon. Further studies are in progress to isolate potent active constituents responsible for uterotonic activity.

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