

## Effect of aqueous extract of *Quercus Infectoria gall* on the basic contractility, frequency and strength of isolated virgin rat uterus smooth muscle

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### ABSTRACT

Myometrial contractions have important role in physiological processes such as sperm and embryo transport, implantation, uterus retraction and reducing post-partum hemorrhage. *Quercus infectoria* (Aleppo oak) is well-known since ancient times as an astringent agent. In the present study, the cumulative effect of the aqueous extract of *Quercus. infectoria* gall (Qig) on the frequency, strength and basic uterine smooth muscle contractility of virgin rats were studied and its contractile effect compared to acetylcholine. This experimental study was carried out on 12 strips taken from the middle part of the uterine of rats (wistar, weight 200-250 g). In the experiments, the cumulative effect of the aqueous extract of Qig on the activity of rat uterine muscle was studied. The cumulative effect of different concentrations of the aqueous extract of Qig significantly increased the contractile response of uterine smooth muscle in a dose-dependent manner. This effect was less than the acetylcholine effect. The aqueous extract of Qig could have potentials for the treatment of disorders associated with low myometrial contractions such as post-partum hemorrhages. In this study, we have proved the high potential of the Qig extract on phasic contraction activity of rat uterus and also the effective dose of extract was identified.

**Keywords:** Aqueous extract, isolated tissue, *Quercus infectoria* gall, rat, uterus smooth muscle

## INTRODUCTION

The myometrium is a phasic smooth muscle with intrinsic ability to generate spontaneous action potentials such as extrinsic neural or hormonal stimuli which are not required for the action potentials to occur but can be modulated by them. The myometrial contractions have important role in physiological processes such as sperm and embryo transport, implantation, uterus retraction and reducing post-partum hemorrhage [1-3]. Disruption of the normal frequency, strength and direction of uterine contractions is associated with a number of female reproductive pathologies including endometriosis and adenomyosis, non-implantation of fertilized embryos during the receptive phase and reduced sperm transport and post-partum hemorrhage [2, 4].

Uterotonic agents are usually employed in the modulation of uterine contractility. Majority of synthetic uterotonic drugs have side effects. Therefore, development of cheaper and safer uterotonic agents with higher efficacy, bioavailability and fewer side effects is an important research goal [5].

Since the ancient times uterotonic plants which stimulate uterine contraction have been used to induce labor, remove the

retained placenta and control of post-partum bleeding and abortion [6].

One of the plants that is popularly used in the southeast Asian to induce uterine contraction is *Quercus infectoria* Olive (Aleppo oak) which called māzūj. The oak tree mainly found in Iran, Anatolia, Syria and Greece [7, 8]. The galls consist of active chemical constituents of oaks were used extensively for medicinal purposes. In fact the *Quercus Infectoria* galls (Qig) is not a type of fruit or any part of a tree by abnormal growth of the oak tree that reacts to a perforation made in the bark of the young twigs by the female gall wasp, cynips gallae-tinctoria, for the purpose of depositing its egg [9].

Qig is consisted of a large amount of tannins (50%, 70%), gallic acid, syringic acid, ellagic acid, sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, methyloleanate and hexagalloyl glucose [10].

Qig has been used as an astringent, an anti-inflammatory agent, an antiseptic and antidiarrheal agent. The Arabs, Persians, Indians, Malays and Chinese have traditionally used the galls of Qig after childbirth to treat vaginal discharge, related postpartum infections and restore uterine elasticity [10, 11].

Experimental studies on Qig in recent years have been reported the diverse pharmacological effect such as antidiarrheal [12], antibacterial [13], larvicidal [14], anti-inflammatory [15], wound and ulcers healing [16], analgesic [11] antiviral, antifungal and anti-amoebic.

In the present study, the cumulative effect of the aqueous extract of Qig on the strength, frequency, and contraction activity of virgin rat uterus were studied. Since the Acetylcholine is one of the strong contraction in rat uterus with the effect on muscarinic receptors, the contractile responses of extract compare with acetylcholine as a power contractile drug were investigated.

## **MATERIALS AND METHODS**

### **Preparation of Qig aqueous extract**

The galls of Qig in this study were collected from the forest around Khoramabad city, Lorestan province in western of Iran. [17]. The taxonomic identification of the plant material was confirmed by center of agriculture and natural resources, Kashan, Iran.

30 g of the galls of Qig were grinded and soaked in 500 mL of distilled water at room temperature for 3 days. Then the mixture was

filtered and evaporated in vacuum to achieve a concentration of 26.6 % .

### **Uterine tissue preparation and *invitro* contraction measurement**

Adult virgin female Wistar rats weighting 200-250 g were obtained from the laboratory animal unit in physiology research center at Kashan university of medical sciences. The rats were housed at a constant temperature and humidity, under light–dark cycle. Vaginal smears were obtained daily from experiment rats, if cytology of smear showed estrous cycle then rats were sacrificed by cervical dislocation.

After midline incision, both uterine horns were surgically removed and placed vertically in a petri dish containing Tyrod solution, a solution that is roughly isotonic with interstitial fluid and used in physiological experiments and tissue culture. This solution composed of: NaCl, 136.9; KCl, 2.68; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 1.05; NaHCO<sub>3</sub>, 11.9; NaH<sub>2</sub>PO<sub>4</sub> 0.42 and glucose 5.55 mM in distilled water. After removing the adherent fat and connective tissues, the middle part of each uterine horn was cut into 10 mm strips in length and opened along the mesometrial border.

Upper end of the tissue strip was tied by a thin cotton thread to a fixed support and the other to an isometric force transducer (Panlab s.l. TR1202p, Spain) coupled to an amplifier (Panlab BR4720, Spain) that linked to a computer data analysis system.

Each tissue holder put in a 25ml organ bath containing Tyrod solution and was aerated with a mixture of oxygen and carbon dioxide at a constant temperature of 37 °C. The Tyrod solution was changed every 10 min. The equilibration period was not less than 30 min, and then spontaneous uterine contractions measured after the amplitude became stable and were taken as the basal value.

After equilibration different concentrations of aqueous extract (0.057, 0.125, 0.25, 0.5, 1 and 2 mg/ml), ach ( $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$ ,  $10^{-3}$  M) and vehicle were cumulatively added to the tissue bath during 60 min.

The contractile response was analyzed by using three parameters: strength of myometrial contractions, the distance between the peak and the initial baseline of the contractions in a period of 10 min after the application of the extract or drug, frequency of myometrial contractions, the number of contractions occurring during a 10 min period after the application of the extract or drug, contraction activity of uterus, the

area under the contractility curve from the start of the induced contraction up to 10 min following the application of the extract or drug.

### **Statistical analysis**

During 60 min with 10 min intervals after drug administration, frequency, duration and area under the curve were measured and expressed as a percentage response relative to the basal activity. All of the data were expressed by ANOVA analysis with statistical significance ( $P < 0.05$ ).

## **RESULTS**

### **Determine the dose of extract**

Tracing of phasic uterine contraction with various doses of Qig aqueous extract (0.001, 0.01, 0.1, 1, 10, 100, 1000, 10000 µg/ml) indicated that force of contraction increases. 1000 µg/ml of extract produced the maximum tension and adding 10000 µg/ml of extract had no significant increase on contraction of uterus. Based on the results of the pilot scheme, concentrations ranging between 10 to 1000 µg/ml extract has been used for study of cumulative effect of Qig aqueous extract on phasic uterus contractions (Figure 1).

**Dose-dependent effect of Qig aqueous extract on strength of uterus contraction isolated from virgin rat**

By cumulative adding of 0.057, 0.125, 0.25, 0.5, 1 and 2 mg/ml of aqueous extract of Qig significantly induced a dose- dependent increase in the spontaneous contractions of

the isolated rat uterus compared to basal activity ( $E_{max} = 152.2 \pm 5.5\%$ ). However, there were no significant different in the control group ( $P < 0.05$ , Figure 2).

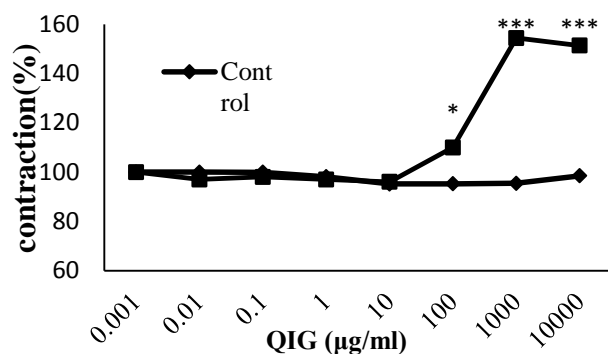


Figure 1. Dose- response curve following adding (0.01, 0.1, 1 ·10· 100· 1000· 10000 µg/ml) QIG aqueous extract

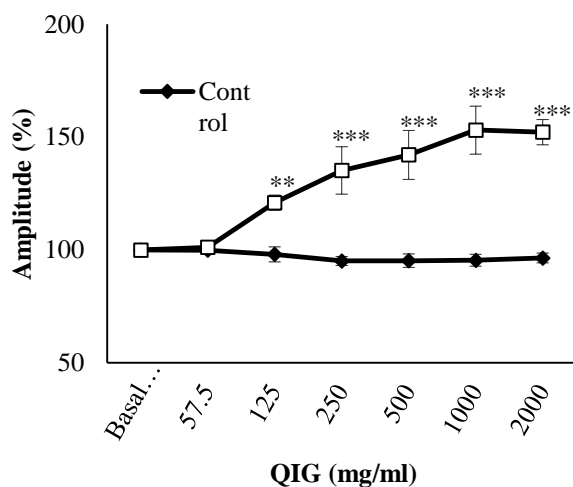


Figure 2. The effect of QIG on strength (amplitude) of uterine contraction isolated from virgin rat

### **Dose-dependent effect of Qig aqueous extract on frequency of uterus contraction isolated from virgin rat**

Following cumulative adding 0.057, 0.125, 0.25, 0.5, 1 and 2 mg/ml of aqueous extract of Qig significantly induced a dose dependent increase in the frequency of the spontaneous contractions of the isolated rat uterus compared to basal activity ( $E_{max}=165.4\pm 20.8\%$ ). However, there were no significant different at the control group (Figure 3).

### **Dose-dependent effect of Qig aqueous extract on contraction activity of uterus (area under the contractility curve) isolated from virgin rat**

Following cumulative adding 0.057, 0.125, 0.25, 0.5, 1 and 2 mg/ml of aqueous extract of Qig significantly induced a dose-dependent increase in the spontaneous contraction activity of the isolated rat uterus compared to basal activity ( $E_{max}=241.1\pm 11.8\%$ ). However, there were no significant different in the control group (Figure 4).

### **Comparing dose-dependent effect of Qig aqueous extract and acetylcholine on strength of uterus contraction isolated from virgin rat**

Following cumulative adding 57.5, 125, 250, 500, 1000 and 2000  $\mu\text{g/ml}$  of aqueous extract of Qig increased the strength of the spontaneous contractions to  $152.2\pm 5.5\%$  at dose dependent manner and cumulative adding  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$ ,  $10^{-3}$  M of acetylcholine which significantly increased strength of the spontaneous contractions. The test also revealed that  $1 \times 10^{-4}$  M Ach produced maximum force of contraction  $E_{max}=138.5\pm 6.8\%$  (Figure 5).

## **DISCUSSION**

The traditional use of plants by women to restoring of health, to tone of uterus and for the induction of labor and abortion suggests that some herbs might be potent uterine stimulants. Therefore, studies of such herbs could provide a helpful guide to the discovery of uterotonic drug. *Qig* extract as an astringent is used for induction of labor and abortion, recovery of the reproductive functions of uterus in postpartum period, and increase the tone and vigor of the vagina in the traditional medicine [9].

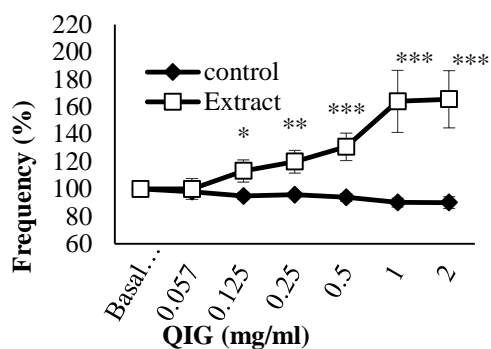


Figure 3. The effect of QIG on frequency of uterine contraction isolated from virgin rat

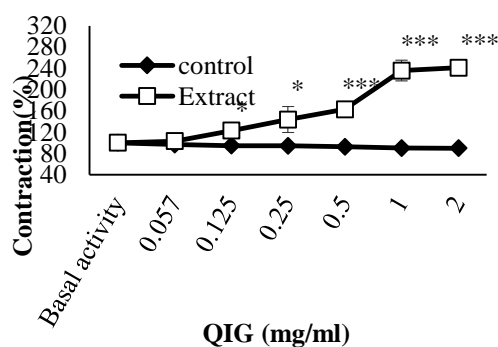


Figure 4. The effect of QIG on contraction activity (area under the curve) of uterine isolated from virgin rat

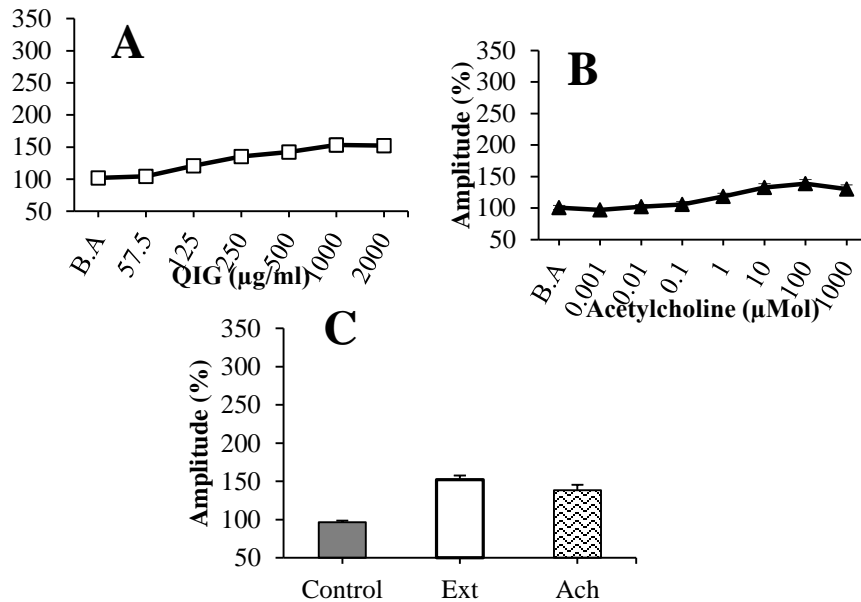


Figure 5. The Comparing effect of QIG and acetylcholine on strength (amplitude) of uterus isolated from virgin rat

Phytochemical analysis of the extract showed presence of flavonoids, tannins, alkaloids, and glycosides in the aqueous extract of *Qig* that are responsible for the plant's effects [8].

The present study revealed that *Qig* extract, in a concentration-dependent manner, increased spontaneous uterine contraction in rat by increasing the contraction amplitude and frequency by 152.2 and 165.4%, respectively.

These findings justify the traditional use of these plants to induce uterine contraction in traditional medical practice.

In contrast to our findings, Thaina [18] and Sireeratawong [19] have reported the spasmolytic activities of *Qig* extract in rat and guinea pig intestinal tissues probably via inhibit the influx of extracellular calcium, which support antidiarrheal and anti-dysentery use of *Qig* extracts in traditional medicine.

Although we have no direct evidence, According to difference in the extractive methods (water, alcohol) and the results of phytochemical screening of extract that showed the presence of variable values of compounds (tannins, alkaloids, flavonoids, and phenolic compounds) in various extracts [8] these constituents may mediate the various effect on tissues. On the other hand,

to exist different receptors in various tissues may cause varied response by similar extract.

Rat uterus is also supplied by parasympathetic nerves, which contribute to the control of muscular activity, so in the following, to assess whether this contractions is useful, the response of the *Qig* aqueous extract was compared with that of Acetylcholine which has the capacity for strong contraction. Addition of increasing concentrations of Ach dose dependently enhanced the contraction activity of the uterine muscles (amplitude and frequency), in a dos dependent manner. This results compared with extract and indicated that extract was equally potent at inducing amplitude of contraction but Ach was more potent (twofold) at increasing frequency of contraction, so, cumulative concentrations of Acetylcholine were increased contraction activity of uterine (area under the contractility curve) more than the extract (1.3 fold). However acute and chronic toxicity studies by Iminjan [10] indicated that aqueous extract of *Qig* is unlikely to have significant toxicity up to a dose level of 2 g/kg of body weight, meanwhile in our study the maximum applied concentration (2000 µg/g) which is roughly equivalent to that reported, so this results can confirm the safety of this extract for uterus contraction.



## CONCLUSION

In this study we have proved for the first time the high potential of the galls of *Q. infectoria* extract to phasic contraction activity of rat uterus and also the effective dose of extract was identified for the first time. It also provides an insight into the usage of these galls in traditional treatment of restore postpartum uterine elasticity. Besides, it can be used effectively as a supplementary agent in clinical treatment of post-partum Hemorrhage. However, further investigations are needed to study in detail the effect of the gall extracts on different part of uterus, to identify the active compounds present in this extract and to elucidate the mechanisms involved in its contractility.

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