

## PHARMACOLOGICAL SCREENING OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI* SEED ON ISOLATED SMOOTH MUSCLE STRIP AND HEART

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Received: 16 March 2012, Revised and Accepted: 20 April 2012

### ABSTRACT

*Syzygium* species are well known plants in the traditional system of Indian medicine with valuable medicinal properties like anti-hyperglycemic, anti-bacterial, hepato-protective, anti-diabetic, anti-inflammatory, chemopreventive, diuretic, etc. In our study, cumulative administration (936.55 µg/ml) of the ethanolic extract of *Syzygium cumini* seed relaxed (55 %) the rat uterus smooth muscle against 22.36 mmol/l of Potassium chloride (KCl) induced smooth muscle contraction. Also, the inotropic and chronotropic effects of the extract using Langendorff's isolated heart perfusion method are reported.

**Keywords:** *Syzygium cumini*, Isolated uterus, Langendorff's isolated heart perfusion method.

### INTRODUCTION

*Syzygium cumini* (Sc) or *Eugenia jambolana* (Myrtaceae) is a well known medicinal plant since ancient times<sup>1</sup>. The ethanolic seed extract has been shown to constitute phytochemical components like alkaloids, glycosides, triterpenoids, carbohydrates, saponins, tannins, flavonoids, phytosterols, amino acids and few phenolic components<sup>2</sup>. The various parts of the plant are pharmacologically proven to possess several properties like anti-inflammatory<sup>3</sup>, chemopreventive<sup>4</sup>, anti-bacterial<sup>5</sup>, diuretic<sup>6</sup>, anti-hyperglycemic<sup>7</sup>, hepatoprotective<sup>8</sup>, and anti-diabetic<sup>9</sup>. Hence, the present study has been made to evaluate the effect of the seed extract on Potassium chloride (KCl) induced contraction in the rat's isolated uterus and heart.

### MATERIALS AND METHODS

#### Plant collection and extraction

The mature fruits of *Syzygium cumini* were collected from a local market in Thanjavur and the seeds were separated from the fruits. The seeds were identified and authenticated by Dr. N. Ravichandran, CARISM, SASTRA University. The seeds were shade dried and coarsely powdered. The seed powder (500 g) was soaked in 95% ethanol and was allowed to remain in an air tight container for about 3 days with continuous agitation of the container to facilitate extraction. The solvent was then filtered and was allowed to evaporate at a controlled temperature (50-60°C). This procedure was repeated several times to obtain the extract which was finally stored in an airtight glass container at 2-8 °C for further experiments.

#### Experimental Animals

Adult healthy Wistar albino female rats (*Rattus norvegicus*) each around 200 g in weight were procured from the Central Animal facility, SASTRA University and were used for the study. Protocols approved by the Institutional Animal Ethics committee (IAEC) were used for the animal experiments. The animals were caged in clean polypropylene cages and water and standard animal diet were given *ad libitum*.

#### Isolated tissue preparation

The rat was sacrificed as per the CPCSEA guidelines. The abdominal cavity was opened and the uterine horn was quickly separated and placed in a Petri dish containing De-Jalon solution (Composition (Per 1000 ml): Sodium chloride: 9.0 g, Potassium chloride: 0.350 g, Calcium chloride: 0.003 g, Glucose: 0.5 g, Sodium bicarbonate: 0.5 g). It was made free from the adhering adipose and connective tissue and was then maintained in 30ml inner organ bath containing De-Jalon solution which was simultaneously aerated with oxygen. The

bath temperature was maintained at 36°C to prevent the development of spontaneous contractions. One end of the tissue was tied to the aeration tube cum tissue holder and the other end was tied to the isotonic frontal writing lever. The tissue was allowed to stabilize for 30 minutes during which the organ bath was well rinsed every 10 minutes. A smoked sheet wrapped around a cylindrical drum in a kymograph instrument (INCO AMBALA, India) was placed in contact with the lever to record the responses produced by the muscle strip.

#### Langendorff's isolated heart perfusion method

The assembly was setup and the animal was sacrificed according to the CPCSEA guidelines. The skin was quickly incised at the midline over the sternum and the heart was exposed by cutting the pericardium. It was quickly isolated from the body along with the aorta and was cannulated immediately. In order to prevent clot formation, the ventricle was gently massaged in Krebs's solution (Composition (Per 1000 ml): Sodium chloride: 6.9 g, Potassium chloride: 0.36 g, Calcium chloride: 0.28 g, Potassium dihydrogen phosphate: 0.16 g, Magnesium sulphate: 0.29 g, Glucose: 2.0 g, Sodium bicarbonate: 2.1 g) to pump out the residual blood. A palmer clip was fixed at the apex of the heart and a thread tied to the hook and passed over pulleys was connected to the recording system. The tension of the lever was adjusted such that it gives maximum contraction. The responses produced by the tissue were recorded in the kymograph instrument (INCO AMBALA, India). The extract was added and the respective heart rate and force of contraction were measured with time.

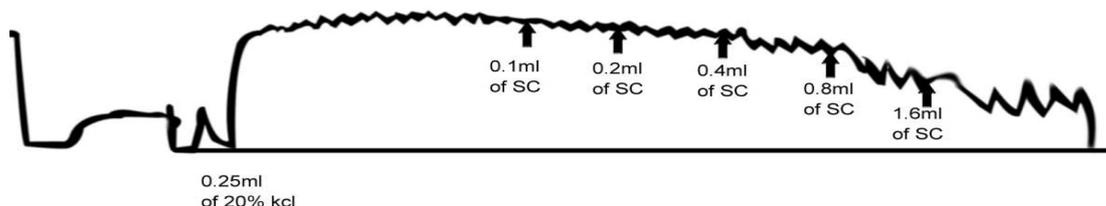
### RESULTS

In the isolated uterus preparation, KCl showed maximum contraction in the uterus smooth muscle strip during which the peak raised up to 2.7 cm from the base line. The same trend prevailed until the muscle strip was exposed to Sc extract. Upon cumulative administration of the seed extract of Sc at an interval of 2 minutes, the peak gradually descended and advanced towards the base line (Fig. 1). The maximum inhibition in contraction was 55% against 100% KCl induced contraction (Table 1). The IC<sub>50</sub> value was obtained using the plot of log concentration of the extract against % inhibition (Fig. 2). The study of isolated rat heart using Langendorff's method showed dose dependent increase in the contraction rate of the heart after the administration of Sc (Table 2). Upon addition of 4 mg/ml of Sc to the isolated heart, there was a significant increase in the number of beats in the isolated heart (42.85 %). This effect continued even upon the addition of 8 mg/ml of Sc, where the heart rate increased two fold (60 %) (Table 2). However, there was no remarkable increase in the force of contraction of the heart muscles upon increasing doses of administration of Sc.

**Table 1: Responses produced by the cumulative addition of *Sc* upon KCl induced contraction of isolated rat uterus**

Volume of the drug (ml)	Dose (mg)	Length (cm)	% Inhibition of contraction
0.1	1	2.5	7.40
0.2	2	2.4	11.11
0.4	4	2.3	14.81
0.8	8	1.5	44.44
1.6	16	1.4	55.55

Abbreviations: KCl induced contraction – 2.7 cm (100%). Lengths are obtained as measures from the baseline



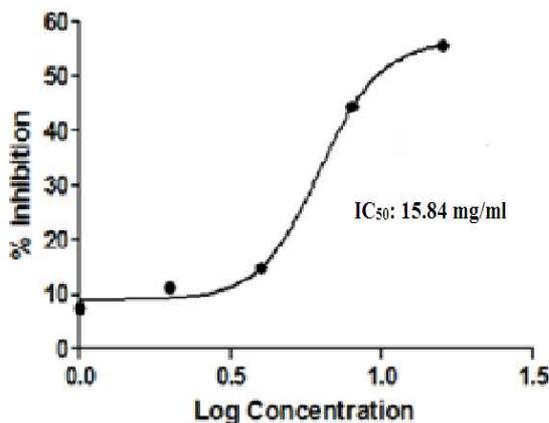
**Fig. 1: Kymograph image produced by the cumulative addition of *Sc* on KCl induced contraction of isolated rat uterus.**

## DISCUSSION

The uterus is a hollow muscular pear-shaped organ composed of three layers of tissue namely perimetrium, myometrium and endometrium. By the years, there has been a tremendous progress towards investigating the physiology of the uterine myometrium. This has given way to various insights for/in understanding conception, contraception and menstrual function<sup>10</sup>. In our study 22.36 mol/l of KCl induces maximum contraction. The possible mechanism behind this would be the extracellular K<sup>+</sup> promoting the influx of Ca<sup>2+</sup> into the cell<sup>11</sup> (Fig. 3). Extracellular potassium ion influences the ATP sensitive potassium channel which leads to an increase in the intracellular potassium ion concentration. This prevents the hyper polarization of the cellular membrane thereby increasing the

cytosolic calcium and by further cell signaling cascades, muscle contraction takes place. The relaxation produced by *Sc* might possibly act via these signaling pathways. This relaxant property of *Sc* reveals the tocolytic effect which prevents the preterm premature rupture of the uterus membrane (PPROM) thereby avoiding premature delivery.

The results obtained in the Langendorff's isolated heart perfusion study showed increased rate of contraction in the heart with no significant change in the force of contraction. Considering smooth muscles, *Sc* can be claimed to possibly act via calcium or potassium channels whereas in case of cardiac muscles the mode of action of *Sc* still remains a question mark. Hence, further investigations are required to positively understand the possible effect of *Sc* on isolated heart.



**Fig. 2: Plot of log concentration of *Sc* extract against percentage inhibition of contraction in isolated uterus muscle strip**

**Table 2: Effect of *Sc* on isolated heart of rat**

Drug treatment	Dose (mg)	Heart rate	% Increase
PSS	--	8	--
<i>Sc</i>	1	8	0.00
<i>Sc</i>	2	10	20.00
<i>Sc</i>	4	14	42.85
<i>Sc</i>	8	20	60.00

Abbreviations: Heart rate obtained for every 15 seconds. PSS- Physiological salt solution- Krebs's

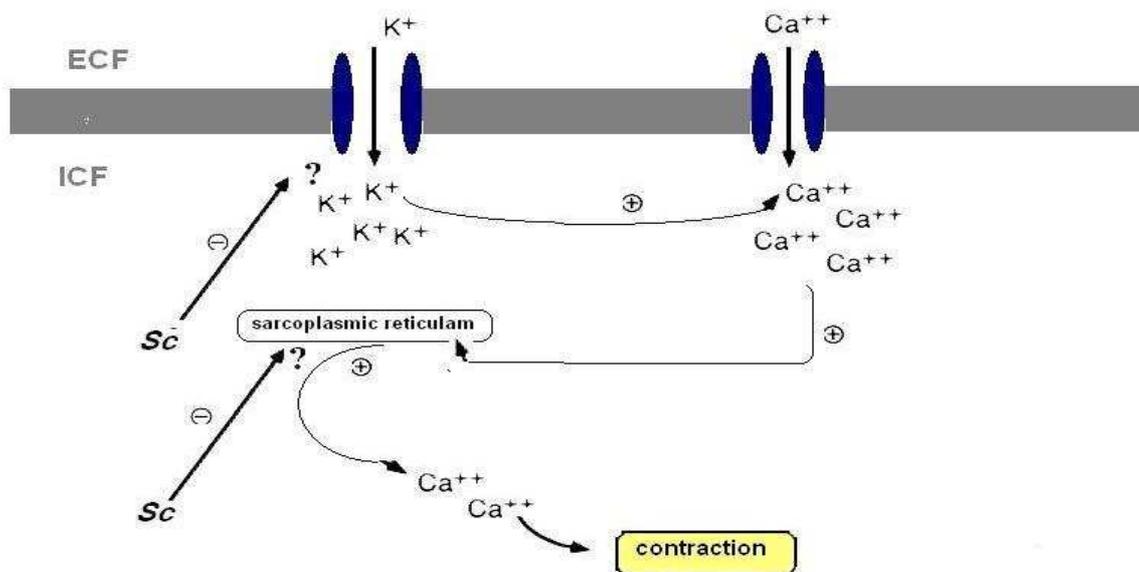


Fig. 3: Mechanism of KCl induced smooth muscle contraction and possible modes of action of Sc

#### CONCLUSION

In our study, the effect of cumulative administration of ethanolic extract of *Syzygium cumini* seeds on the isolated rat uterus and heart has been highlighted. Further investigations have to be carried out for the detailed revelation of the mechanism of the relaxation obtained.

#### ACKNOWLEDGEMENTS

The authors wish to thank the management of SASTRA University for the infrastructure facilities that were provided for performing the experiments.

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