

**EVALUATION OF SKELETAL MUSCLE ACTIVITY OF *OCIMUM SANCTUM* LEAVES  
AND SEEDS EXTRACT ON FROG'S RECTUS ABDOMINUS MUSCLE**

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## 1. INTRODUCTION

### Nomenclature

Tulsi is a sacred plant. *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, holy basil, or tulsi (also spelled tulasi), is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.<sup>[1-2]</sup>

**Description:** It is an erect, many branched subshrub, 30-60 cm (12-24 in) tall with hairy stems and simple phyllotaxic green or purple leaves that are strongly scented. Leaves have petioles and are ovate, up to 5cm (2.0 in) long, usually slightly toothed. The flowers are purplish in elongate racemes in close whorls.<sup>[3-4]</sup>

**Pharmacognostic study:** It is much branched small herb and 30 to 75 cm in height. All parts of tulsi are used in medicine, especially fresh and dried leaves. Leaves are oblong, acute with entire or serrate margin, pubescent on both sides and minutely gland dotted. The leaves are green in color with aromatic flavor and slightly pungent taste. Flowers are purplish in color in the form of racemes. Nutlets are subglobose, slightly compressed, pale brown or red in color. Seeds are reddishblack and subglobose.<sup>[5]</sup>

**Cultivation:** Tulsi grows up to 30-130cm tall, with opposite, light green, silky leaves 3-11cm long and 1-6cm broad. The flowers are small, white in color and are arranged in a terminal spike. Unusual among Lamiaceae, the four stamens and the pistil are not pushed under the upper lip of corolla, but lie over the inferior lip. After entomophilous pollination, the corolla falls off and four round achenes develop inside the bilabiate calyx. Tulsi is very sensitive to cold, with best growth in hot, dry conditions. It behaves as an annual if there is any chance of a frost. Although basil will grow best outdoors, it can be grown indoors in a pot and like most herbs, will do best on an equator-facing windowsill. It should be kept away from extremely cold drafts, and grows best in strong sunlight: therefore a greenhouse or row cover is ideal if available. They can however, be grown even in a basement, under fluorescent lights.<sup>[6]</sup>



**Image No. 1: Tulasi Plant.**

### Scientific classification

Kingdom:	Plantae
Family:	Lamiaceae
Genus:	<i>Ocimum</i>
Species:	<i>Tenuiflorum</i>
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Lamiales

Classification of *ocimum sanctum*.

**Uses:** Tulsi is also known as "the elixir of life" since it promotes longevity. Different parts of *Ocimum sanctum* plant are used in Ayurveda and Siddha Systems of Medicine for prevention and cure of many illnesses and everyday ailments like common cold, headache, cough, flu, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic diseases, malaria fever, as an antidote for snake bite and scorpion sting, flatulence, migraine

headaches, fatigue, skin diseases, wound, insomnia, arthritis, digestive disorders, night blindness, diarrhea and influenza. The leaves are good for nerves and to sharpen memory. Chewing of Tulsi leaves also cures ulcers and infections of mouth.<sup>[7-8]</sup>

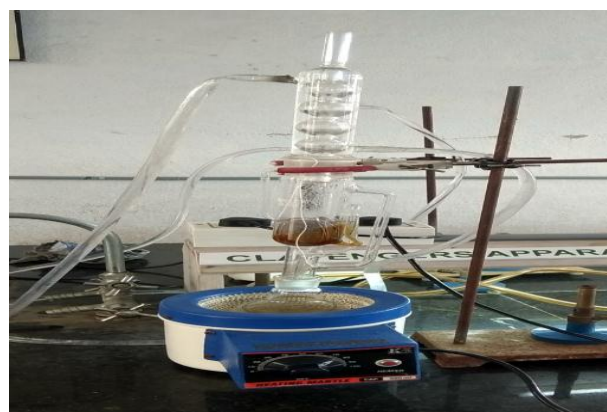
## 2. MATERIALS AND METHODOLOGY

**Collection of plant material:** Tulasi was collected from the botanical garden of Vaageswari institute of pharmaceutical sciences, karimnagar, Telangana.

**Preparation of plant extract:** a) 50 gms of tulsi leaves were obtained and washed. The collected leaves were dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh. Soxhalation with water (aqueous) for 10-20 cycles. the final product was dried and weighed.<sup>[9]</sup>



**Image. 2: Tulasi leaves powder and tulasi leaves extract.**



**Image. 3: Soxhalation of ocimum sanctum.**



**Image. 4: Dissection of frog.**

**Effect of Tulasi Leaves Extract(TLE) on The Skeletal Muscle of The Frog:** Since the antimigraine drugs were reported to have skeletal muscle activity, so this experiment was attempted to assess the effect of tulasi leaves extract on the frog rectus abdominus muscle preparation. The experiment was carried as per the method described by kulkarni.

Frogs weighing 20-25g were used in this study. The frog was stunned and decapitated and the spinalcord was destroyed. A frog was pithed and the skin of the anterior and abdominal wall was cut by a midline incision and then it was cut laterally to expose the anterior abdominal wall. The two rectus were seen running from the base of sternum. The muscles were cut across just above the sternum at its base and the pair of muscles attached to it were dissected and transferred to a dish containing frog ringer solution at room temperature. The muscles were then carefully cleaned and one of them was trimmed to the desired size and mounted in an organ bath filled with ringer solution at room temperature and aerated by stream of fine bubbles emerging near the bottom of the bath. Isotonic concentrations were recorded using gimbel lever with a sideways writing point. The lever was balanced for a tension of approximately 2-5g. An extra load of approximately 1g on the long arm was supplied because sometimes the lever may not return to the base line after washing. The drug period allowed for stabilization was 30 minutes during which the muscle was subjected to 1g stretch. At 0<sup>th</sup> min –the kymograph was started after raising the extra load; in the 1<sup>st</sup> min-the drug was added and in the 2<sup>nd</sup> min-the kymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5<sup>th</sup> min- the lever point was brought to the base line and the next cycle was started. After recording the graded responses to different log dose of acetylcholine, the Tulasi leaves extract was added and its effects upon acetylcholine induced contractions as well as the effect of its own in the tissue was studied.

(b): Tulasi seeds were collected from the botanical garden of vaageswari institute of pharmaceutical sciences, Karimnagar, Telangana.

**Preparation of Tulasi Seeds Extract:** 50 gms of tulasi seeds were obtained and washed. The collected Seeds were dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh .soxhalation with distilled water (aqueous)for 10-20 cycles. The final product was dried and weighed.



**Image No. 5: Tulasi seeds.**

**Effect of Tulasi Seeds Extract (Tse) on The Skeletal Muscle of the Frog:** Since the antimigraine drugs were reported to have skeletal muscle activity, so this experiment was attempted to assess the effect of tulasi Seeds extract on the frog rectus abdominus muscle preparation. The experiment was carried as per the method described by kulkarni. Frogs weighing 20-25g were used in this study. The frog was stunned and decapitated and the spinalcord was destroyed. A frog was pithed and the skin of the anterior and abdominal wall

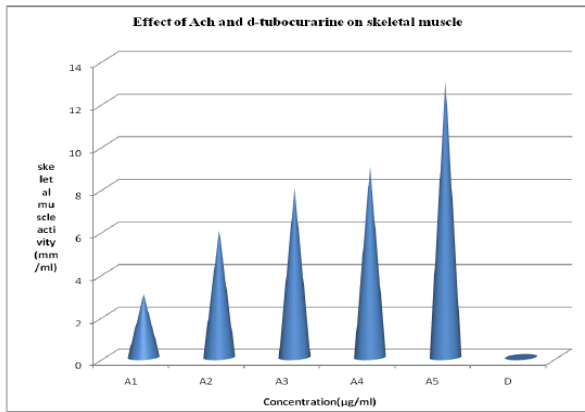
was cut by a midline incision and then it was cut laterally to expose the anterior abdominal wall. The two rectus were seen running from the base of sternum. The muscles were cut across just above the sternum at its base and the pair of muscles attached to it were dissected and transferred to a dish containing frog ringer solution at room temperature. The muscles were then carefully cleaned and one of them was trimmed to the desired size and mounted in an organ bath filled with ringer solution at room temperature and aerated by stream of fine bubbles emerging near the bottom of the bath. Isotonic concentrations were recorded using gimbel lever with a sideways writing point. The lever was balanced for a tension of approximately 2-5g. An extra load of approximately 1g on the long arm was supplied because sometimes the lever may not return to the base line after washing. The drug period allowed for stabilization was 30 minutes during which the muscle was subjected to 1g stretch. At 0<sup>th</sup> min –the kymograph was started after raising the extra load; in the 1<sup>st</sup>min-the drug was added and in the 2<sup>nd</sup> min-the kymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5<sup>th</sup> min- the lever point was brought to the base line and the next cycle was started. After recording the graded responses to different log dose of acetylcholine, the Tulasi Seedes extract was added and its effects upon acetylcholine induced contractions as well as the effect of its own in the tissue was studied.<sup>[10-13]</sup>

### 3. RESULTS

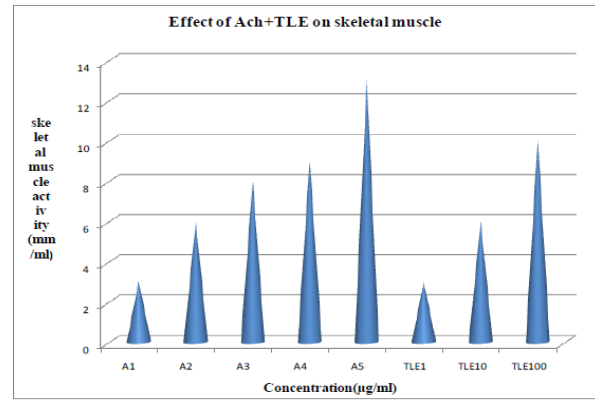
**Table. 1: Skeletal muscle activity of acetylcholine, TSE and TLE, D-tubocurarine, Acetylcholine +TSE, Acetylcholine + TLE.**

S. No	Drug	Dose(µg/ml)	Height(mm)	Response
1	Acetylcholine	1	3	Increased
2	Acetylcholine	2	6	Increased
3	Acetylcholine	4	8	Increased
4	Acetylcholine	8	9	Increased
5	Acetylcholine	16	13	Increased
6	d-tubocurarine	4	-	-
7	TLE	1	3	Increased
8	TLE	10	6	Increased
9	TLE	100	10	Increased
10	TSE	1	3	Increased
11	TSE	10	5	Increased
12	TSE	100	9	Increased
13	Acetylcholine+ TLE	1 1	5	Increased
14	Acetylcholine+ TLE	1 10	8	Increased
15	acetylcholine+ TLE	1 100	13	Increased
16	Acetylcholine+ TSE	1 1	5	Increased
17	Acetylcholine+ TSE	1 10	9	Increased
18	Acetylcholine+ TSE	1 100	13	Increased

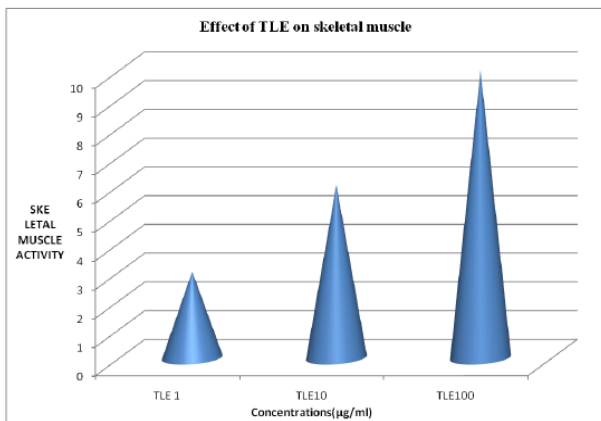




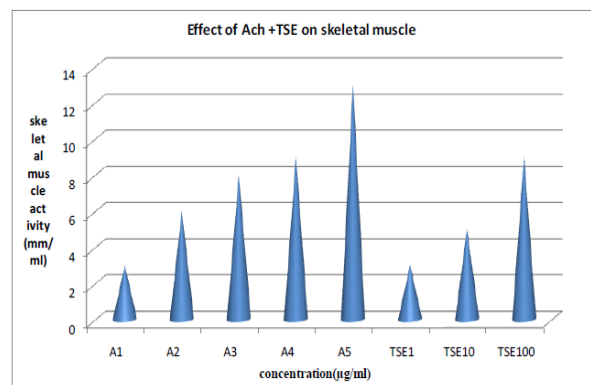
**Figure No. 1: Effect of Ach and d-tubocurarine on skeletal muscle.**  
 A1-Acetylcholine-1µg/ml,A2-Acetylcholine-2µg/ml,A3-Acetylcholine-4µg/ml, A4-Acetylcholine-8µg/ml,A5-Acetylcholine-16µg/ml, and D-tubocurarine.



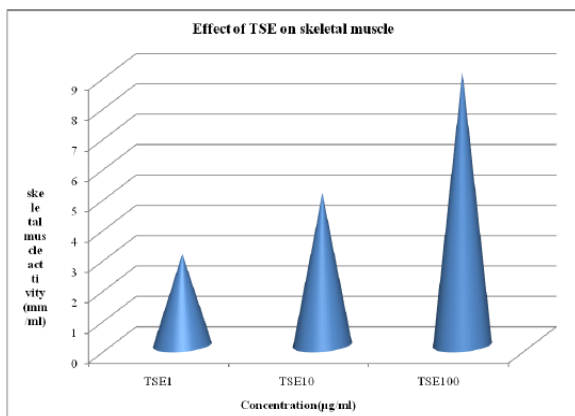
**Figure No. 4: Effect of Ach+TLE on skeletal muscle.**  
 A1: Acetylcholine 1µg/ml, A2:Acetylcholine 2µg/ml,A3.Acetylcholine 4µg/ml, A4: Acetylcholine 8µg/ml,A5:Acetylcholine 16µg/ml,and TLE1(Tulasi leaves Extract): 1µg/ml,TLE:10µg/ml,TLE100µg/ml.



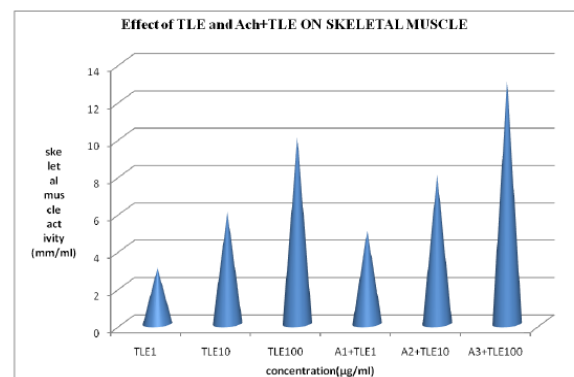
**Figure No. 2: Effect of TLE on skeletal muscle.**  
 TLE1-Tulasi leaves extract 1µg/ml,TLE10-Tulasi leaves extract 10µg/ml, TLE100-Tulai leaves extract 100µg/ml.



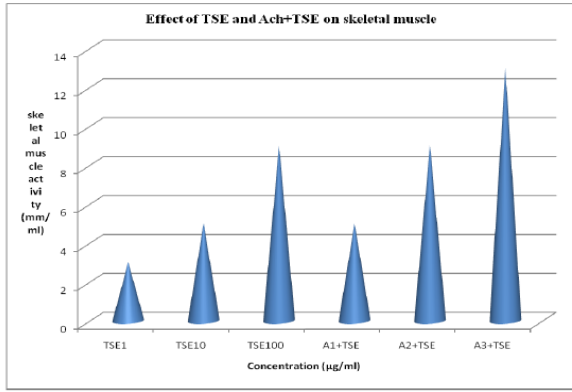
**Figure No. 5: Effect of Ach+ TSE on skeletal muscle.**  
 A1: Acetylcholine 1µg/ml, A2: Acetylcholine 2µg/ml, A3.Acetylcholine 4µg/ml, A4: Acetylcholine 8µg/ml, A5:Acetylcholine 16µg/ml, and TSE1(Tulasi Seeds Extract): 1µg/ml,TSE:10µg/ml,TSE100µg/ml.



**Figure No. 3: Effect of TSE on skeletal muscle.**  
 TSE1: 1µg/ml, TSE2: 10µg/ml, and TSE3: 100µg/ml. (Tulasi Seed Extract).



**Figure No. 6: Effect of TLE and Ach+TLE on skeletal muscle.**  
 TLE1-Tulasi leaves extract 1µg/ml,TLE10-Tulasi leaves extract 10µg/ml, TLE100-Tulai leaves extract 100µg/ml. A1:Aetylcholine1µg/ml+TLE1:1µg/ml, A2: Aetylcholine1µg/ml+ TLE1:10µg/ml,A3: Aetylcholine1µg/ml+ TLE1:100µg/ml.

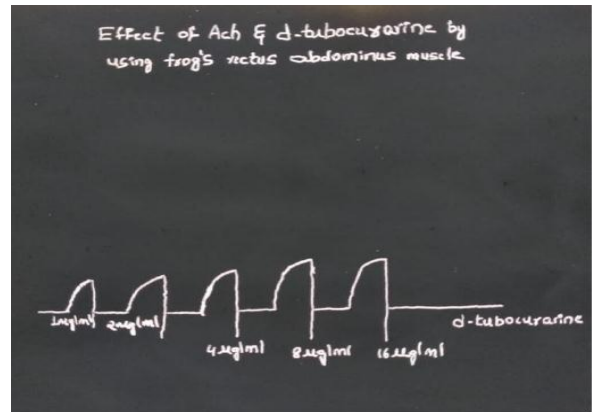


**Figure No. 7: Effect of TSE and Ach+TSE on skeletal muscle** TSE1-Tulasi seeds extract 1µg/ml, TSE10-Tulasi seeds extract 10µg/ml TSE100-Tulasi seeds extract 100µg/ml.

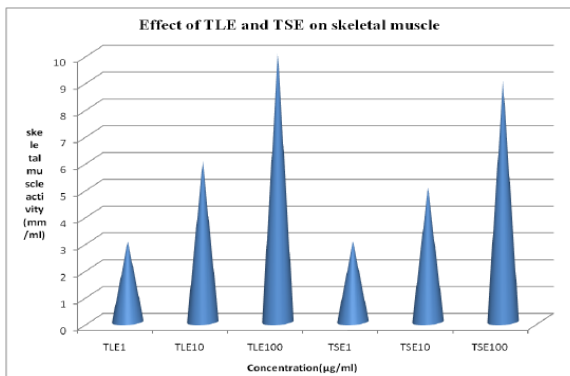
A1: Aetylcholine 1µg/ml+TSE1:1µg/ml,  
Aetylcholine 1µg/ml+ TSE1:10µg/ml,  
Aetylcholine 1µg/ml+ TSE1:100µg/ml

A2:  
A3:

A1: Aetylcholine 1µg/ml+TSE1:1µg/ml, A2:  
Aetylcholine 1µg/ml+ TSE1:10µg/ml, A3:  
Aetylcholine 1µg/ml+ TSE1:100µg/ml (TSE=Tulasi  
Seeds Extract)

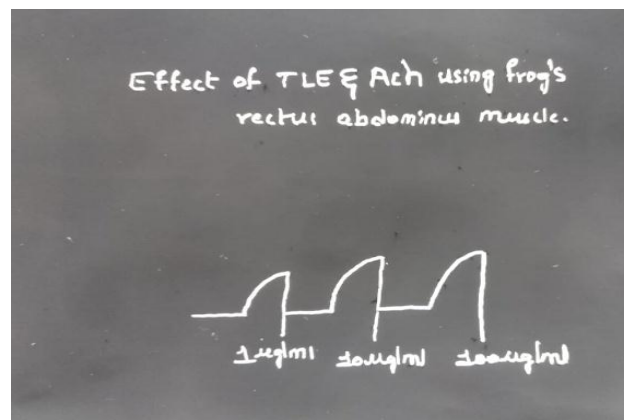
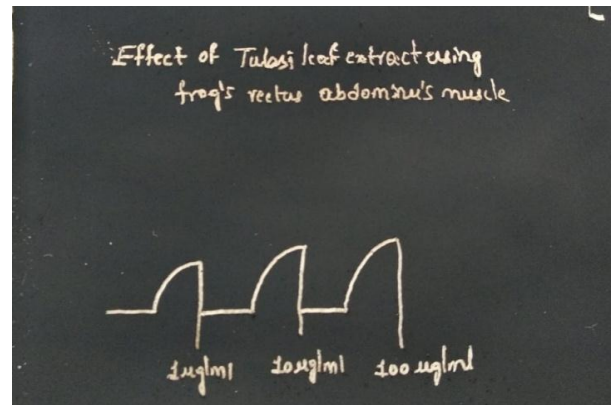


**Image No. 6: Kymograph –Effect of Ach on skeletal muscle.**

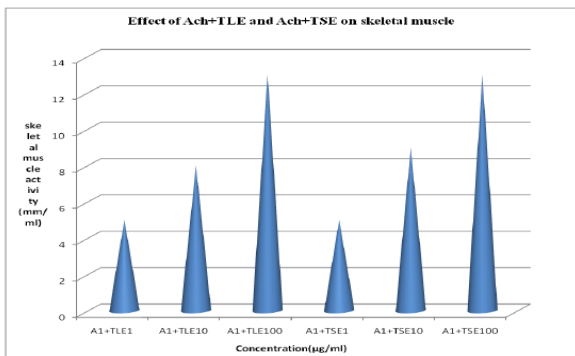


**Figure No. 8: Effect of TLE and TSE on skeletal muscles.**

TLE1-Tulasi leaves extract 1µg/ml, TLE10-Tulasi leaves extract 10µg/ml, TLE100-Tulasi leaves extract 100µg/ml. TSE1:1µg/ml, TSE2:10µg/ml, and TSE3:100µg/ml. (Tulasi Seed Extract)



**Image No. 7: Kymograph –Effect of TLE and TLE+Ach on skeletal muscle.**



**Figure No. 9: Effect of Ach+TLE and Ach+TSE on skeletal muscle.**

A1: Aetylcholine 1µg/ml+TLE1:1µg/ml, A2:  
Aetylcholine 1µg/ml+ TLE1: 10µg/ml, A3:  
Aetylcholine 1µg/ml+ TLE1:100µg/ml (TLE=Tulasi  
Leaves Extract).

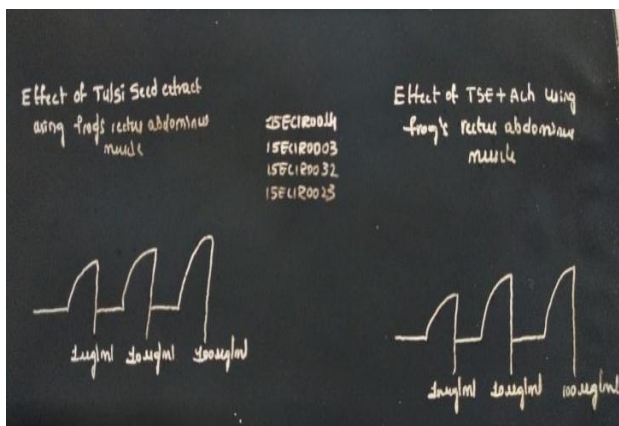


Image No. 8: Kymograph –Effect of TSE and TSE+ACh on skeletal muscle.

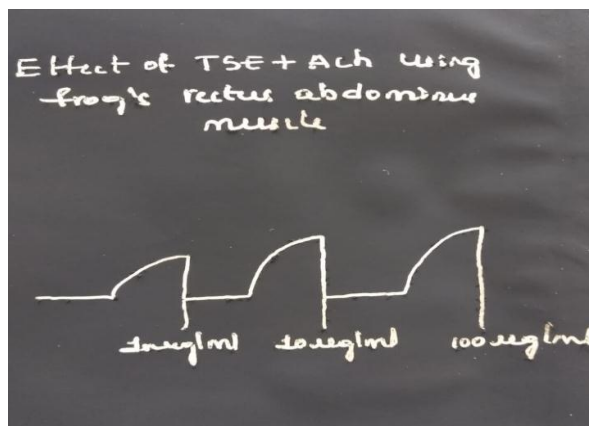


Image No. 10: Kymograph –Effect of TLE+ACh and TSE+ACh on skeletal muscle.

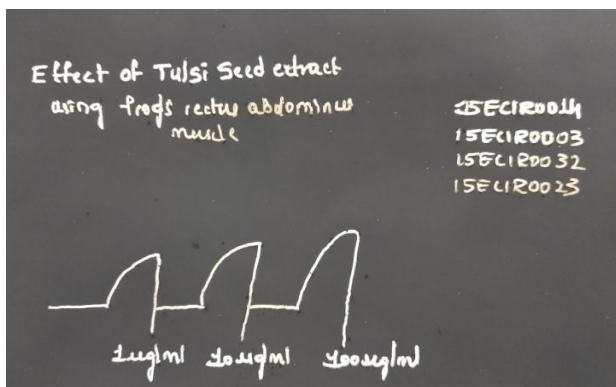
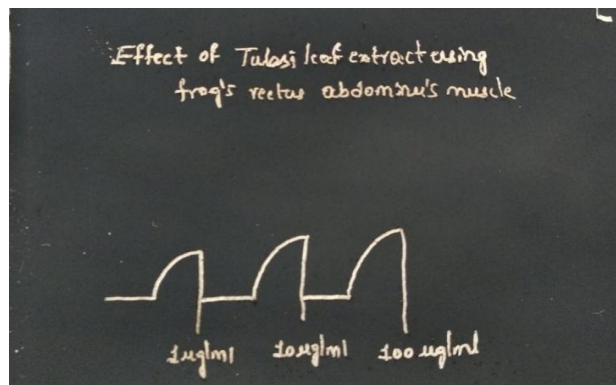
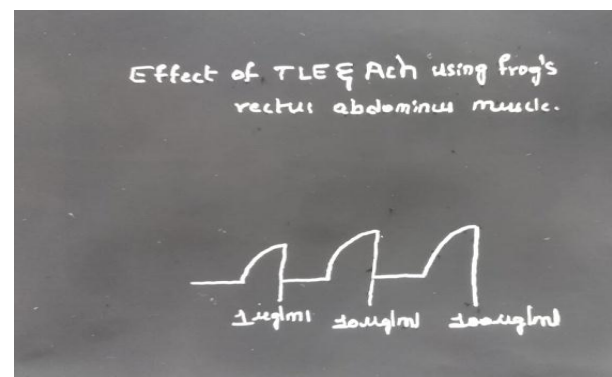


Image No. 9: Kymograph-effect of TLE and TSE on skeletal muscle.



4. DISCUSSION

The tulsi extract was found to have skeletal muscle activity with the concentration of 1µg/ml, 5µg/ml, 10µg/ml.

When the activity was compared between the standard drug i.e, Acetylcholine and test drug Tulsi extract. The activity of the standard drug is more compare to test drugs and it is above to reach with the standard drug.

The skeletal muscle activity was evaluated first by the acetylcholine of different doses like 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, and 8µg/ml and with d-tubocurarine of doses about 16µg/ml. The acetylcholine were shown more activity by increasing the dose response whereas, the drug d-tubocurarine has shown no effect and no action it neither contraction nor depolarization because it inhibits muscular contraction induced by the application of acetylcholine.

Then skeletal muscle activity is evaluated by using test drugs tulsi leaves extract and tulsi seeds extract of using different doses like 1µg/ml, 10µg/ml, and 100µg/ml. For both the tests drugs the response have been increased.

The effect of acetylcholine and TLE and were compared and the result shown the more active response with the acetylcholine rather than the TSE.

The effect of single TSE and combination of TSE +ACh, and effect of single TLE and combination of TLE+ACh is compared and the result shown more active with the combination.

But from both the combination ACh +TSE and ACh +TLE, the better and more active results was shown by the TLE+ACh.

The comparison study between TSE and TLE, the TLE have shown more active response than TSE.

Comparative study between A1+TSE and A1+TLE were studied and the result that the acetylcholine i.e., 1µg/ml +TSE 1µg/ml was less active than acetylcholine

1µg/ml+TSE5 10µg/ml and this is less than acetylcholine 1µg/ml+TSE100 100µg/ml same results is also shown by the TLE by increasing the concentration, the active response is increased.

From both the comparison the maximum active response were shown by the ACH+TLE THAN ACH +TSE.

Thus from present study it was concluded that the Tulasi leaves extract has good skeletal muscle activity than the tulasi seeds extract.

Thus the present investigation proves that TSE and TLE were have good skeletal muscle activity alone and combination with acetylcholine and it produces the significant skeletal muscle activity at higher concentrations.

## 5. CONCLUSION

The tulasi leaves and tulasi seeds extract was found to good skeletal muscle activity with different concentrarions. When the activity was compared between the standard drug i.e, acetylcholine and test drugs the tulasi leaves extract and tulasi seeds extract. The activity of the standard drug is more compare to test drugs and it is above to reach with the standard drug.

The skeletal muscle activity is evaluated by using test drugs tulasi leaves extract of using different doses like 1µg/ml,10µ/ml,100µg/ml. for both the test drugs response have been increased. The effect of acetylcholine and tulasi leaves extract (TLE)were compared and the result show the more active response with the acetylcholine rather than tulasi leaves extract the effect of acetylcholine and tulasi seeds extract were compared and the results shown the more active response with the acetylcholine rather than tulasi seeds extract.

The study finally concluded that the effect of tulasi leaves extract and combination of tulasi leaves extract and acetylcholine is compared and the results shown more active with the combination of tulasi seeds extract and acetylcholine. It was selected for further investigation including bioassay guided fractionation,in order to isolate the constituents responsible for the effect of the plant.

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