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Research Article

EVALUATION OF SKELETAL MUSCLE ACTIVITY OF FENUGREEK SEEDS EXTRACT AND FENUGREEK LEAVES EXTRACT ON ISOLATED FROG'S RECTUS ABDOMINUS MUSCLE

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Skeletal muscle activity of Fenugreek seeds extract and Fenugreek leaves extract were studied in the green frog (Rana hexadactyla) by the rectus abdominis muscle preparation. Fenugreek seeds extract and Fenugreek leaves extract with distilled water 1µg/ml, 10µg/ml and 100µg/ml concentrations. The result indicated that the treatment of Fenugreek seeds extract and Fenugreek leaves extract alone and combination with acetylcholine produce skeletal muscle activity. Thus from the present study it was concluded that Fenugreek seeds extract and Fenugreek leaves extract were have good skeletal muscle activity alone and combination with Acetylcholine. Fenugreek leaves extract have more potent skeletal muscle activity than Fenugreek seeds extract.

Keywords: *Skeletal muscle activity, Fenugreek seeds extract and Fenugreek leaves extract Rana hexadactyla, Acetylcholine.*

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INTRODUCTION:

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family Leguminosae has definite anti hyperglycemic activity. Other pharmacological properties such as anti inflammatory, antioxidant, anti microbial, antiviral, hypotensive, and hypercholesterolemia are also exhibited [1]. The importance of Fenugreek is considered in the presence of metal contents which have a significant role in the biological activity. Chromium a key constituent of the Glucose tolerance factor is vital to maintain proper blood sugar. Chromium supplementation can break the cycle of overeating sweets, at the same time chromium breaks down glucose and fatty acids more efficiently within the body. Most of the herbs contain chromium but highest amount found in Fenugreek [2]. The active constituents involved in producing the above activities are pyridine type alkaloids like gentianine, trigonelline, choline, flavanoids-orientin, vitexin, quercetin; steroidal saponins –diosgenin, yamogenin, and gitogenin.[3-4]. Fenugreek (*Trigonella foenum graecum*), native to southern Europe and Asia, is an erect annual herb with white flowers and hard, yellowish brown

and angular seeds. Known as methi in Hindi, it is a popular ingredient many North Indian dishes.[5]. This present study was to evaluate the skeletal muscle activity of Fenugreek seeds extract and Fenugreek leaves extract on isolated frog's rectus abdominus muscle.

MATERIALS AND METHODS:

Collection of Plant material:

Fenugreek (*Trigonella foenum*) seeds and leaves were collected from the botanical garden of vaageswari institute of pharmaceutical sciences, karimnagar, Telangana.

Preparation of plant extract:

500gm of fenugreek seeds were obtained and washed. The collected seeds were dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh and was macerated with 70% ethanol for 5days. The filtrate was dried, concentrated to dryness invacuum and weighed. 1kg of fenugreek leaves were obtained, washed and dried. The collected dried leaves, pulverized by a mechanical grinder, sieved through 60 mesh and was soxhalation with Ethanol for 5-10 cycles. The final product was dried and weighed.[6]



Fig 1: Maceration for Fenugreek Seeds



Fig 2: Soxhalation for Fenugreek Leaves

Effect of Fenugreek Seeds Extract (FSE) and Fenugreek Leaves Extract (FLE) 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 Fenugreek leaves and seeds extracts on the frog rectus abdominis

muscle preparation. The experiment was carried as per the method described by Kulkarni.[7]

Frogs weighing 20-25 g were used in this study. The frog was stunned and decapitated and the spinal cord 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 contractions 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 sometime 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 0th min - the kymograph was started after raising the extra load; in the 1st min- the drug was added and in the 2nd min- the kymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5th 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 Fenugreek leaves and seeds extracts was added and their effects upon acetylcholine induced contractions as well as the effect of its own in the tissue was studied.[8,9]



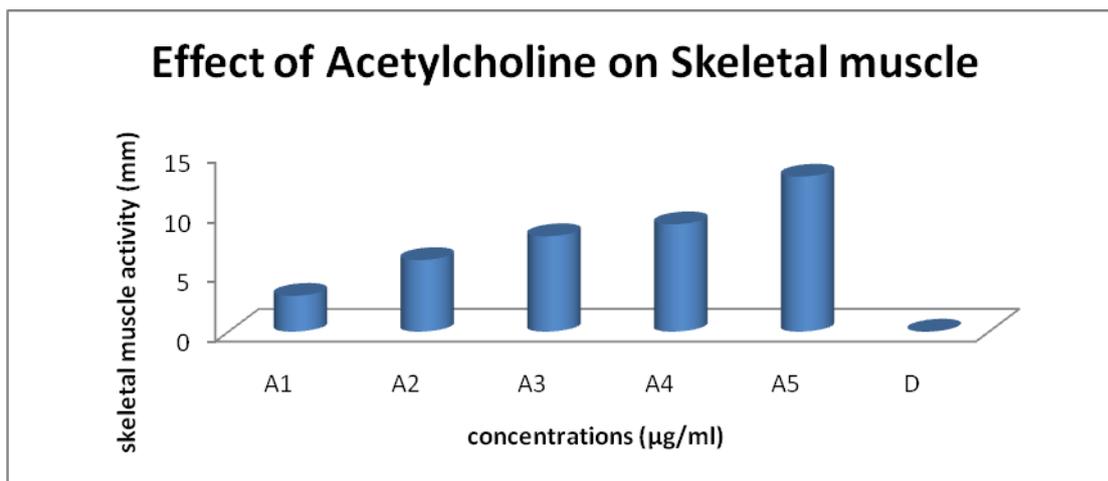
Fig 3: Isolation of Frog's rectus abdominus muscle



Fig 4: Kymograph setup in Pharmacology Lab

RESULTS:**Table 1: Skeletal muscle activity of Acetylcholine, d-Tubocuraine , FSE, FLE, Acetylcholine + FSE and Acetylcholine + FLE.**

| S.NO | DRUG | DOSE ($\mu\text{g/ml}$) | 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-tubocuraine | 4 | - | - |
| 7 | FSE | 1 | 2 | Increased |
| 8 | FSE | 10 | 8 | Increased |
| 9 | FSE | 100 | 10 | Increased |
| 10 | FLE | 1 | 3 | Increased |
| 11 | FLE | 10 | 9 | Increased |
| 12 | FLE | 100 | 13 | Increased |
| 13 | Acetylcholine + FSE | 1 1 | 5 | Increased |
| 14 | Acetylcholine + FSE | 1 10 | 12 | Increased |
| 15 | Acetylcholine + FSE | 1 100 | 16 | Increased |
| 16 | Acetylcholine + FLE | 1 1 | 6 | Increased |
| 17 | Acetylcholine + FLE | 1 10 | 17 | Increased |
| 18 | Acetylcholine + FLE | 1 100 | 19 | Increased |

**Fig 5 : Effect of Acetylcholine on Skeletal muscle**

A1- Acetyl choline-1 $\mu\text{g/ml}$ A2-Acetyl choline-2 $\mu\text{g/ml}$ A3- Acetyl choline-4 $\mu\text{g/ml}$
A4- Acetyl choline-8 $\mu\text{g/ml}$ A5- Acetyl choline-16 $\mu\text{g/ml}$ and D-d-tubocuraine

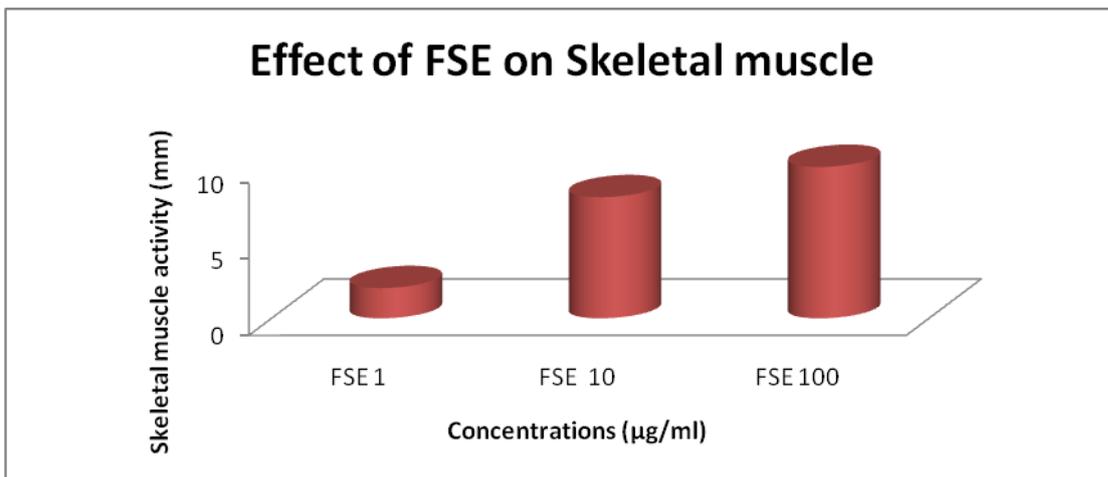


Fig 6: Effect of FSE on Skeletal muscle

FSE1- Fenugreek Seeds Extract 1 $\mu\text{g/ml}$, FSE10- Fenugreek Seeds Extract 10 $\mu\text{g/ml}$, FSE100- Fenugreek Seeds Extract 100 $\mu\text{g/ml}$

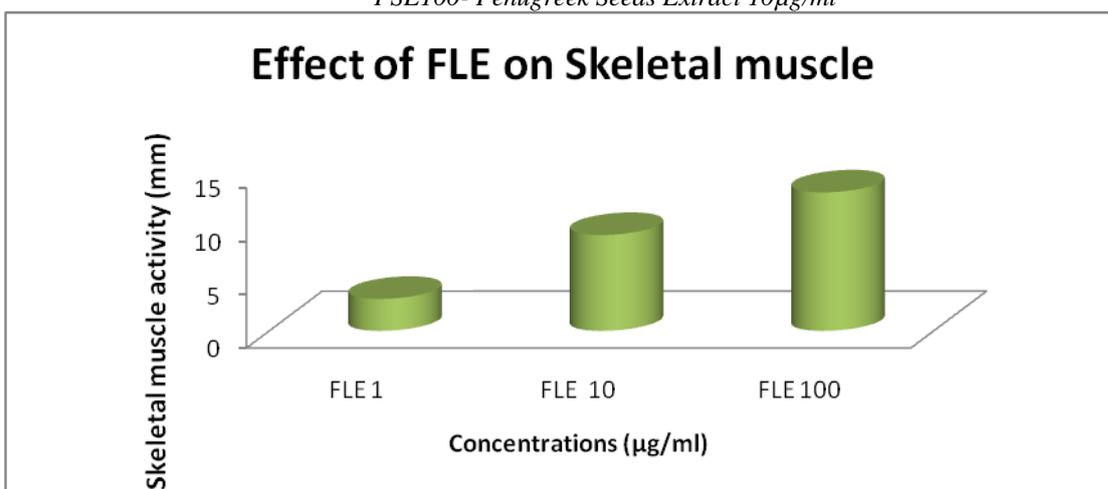


Fig 7: Effect of FLE on Skeletal muscle

FLE1- Fenugreek Leaves Extract 1 $\mu\text{g/ml}$, FLE10- Fenugreek Leaves Seeds Extract 10 $\mu\text{g/ml}$, FLE100- Fenugreek Leaves Extract 100 $\mu\text{g/ml}$

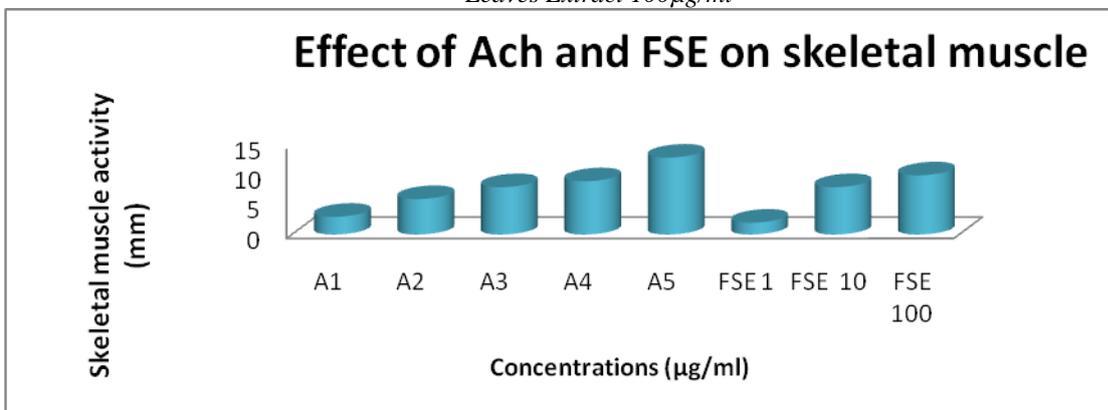


Fig 8: Effect of Ach and FSE on skeletal muscle

A1- Acetyl choline-1 $\mu\text{g/ml}$ A2 -Acetyl choline-2 $\mu\text{g/ml}$ A3-Acetyl choline-4 $\mu\text{g/ml}$ A4- Acetyl choline-8 $\mu\text{g/ml}$ A5- Acetyl choline-16 $\mu\text{g/ml}$, FSE1- Fenugreek Seeds Extract 1 $\mu\text{g/ml}$, FSE10- Fenugreek Seeds Extract 10 $\mu\text{g/ml}$, FSE100- Fenugreek Seeds Extract 100 $\mu\text{g/ml}$

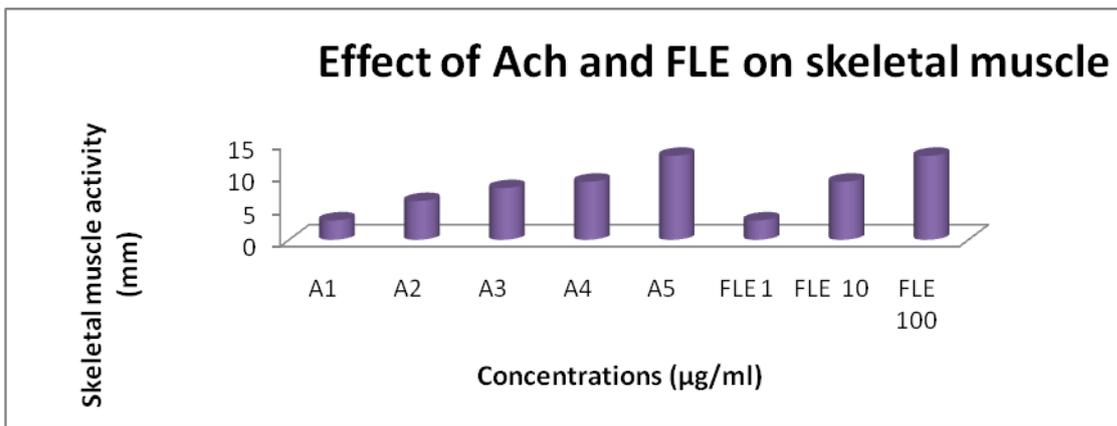


Fig 9: Effect of Ach and FLE on skeletal muscle

A1- Acetyl choline-1µg/ml A2 - Acetyl choline-2µg/ml A3- Acetyl choline-4µg/ml
 A4- Acetyl choline-8µg/ml A5- Acetyl choline-16µg/ml, FLE1-Fenugreek Leaves Extract 1µg/ml , FLE10-
 Fenugreek Leaves Seeds Extract 10µg/ml, FLE100- Fenugreek Leaves Extract 100µg/ml.

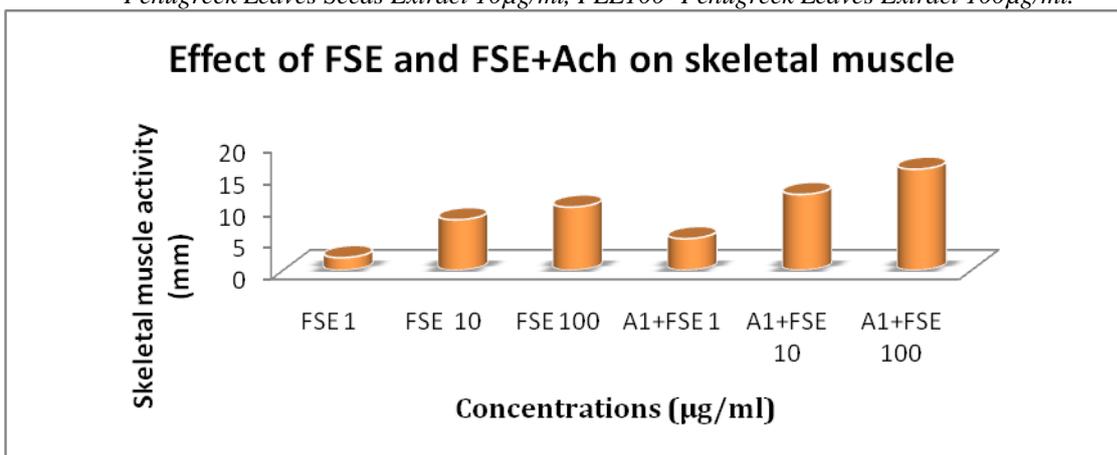


Fig 10: Effect of FSE and FSE+Ach on skeletal muscle

A1- Acetyl choline-1µg/ml, FSE1- Fenugreek Seeds Extract 1µg/ml, FSE10- Fenugreek Seeds Extract 10µg/ml
 FSE100- Fenugreek Seeds Extract 100µg/ml

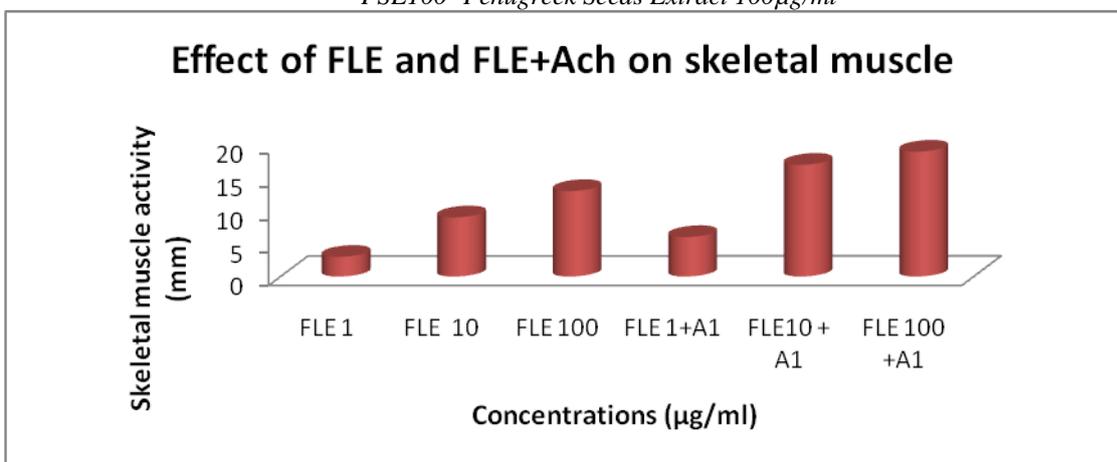


Fig 11: Effect of FLE and FLE+Ach on skeletal muscle

A1- Acetyl choline-1µg/ml, FLE1- Fenugreek Leaves Extract 1µg/ml , FLE10- Fenugreek Leaves Seeds Extract
 10µg/ml, FLE100- Fenugreek Leaves Extract 100µg/ml.

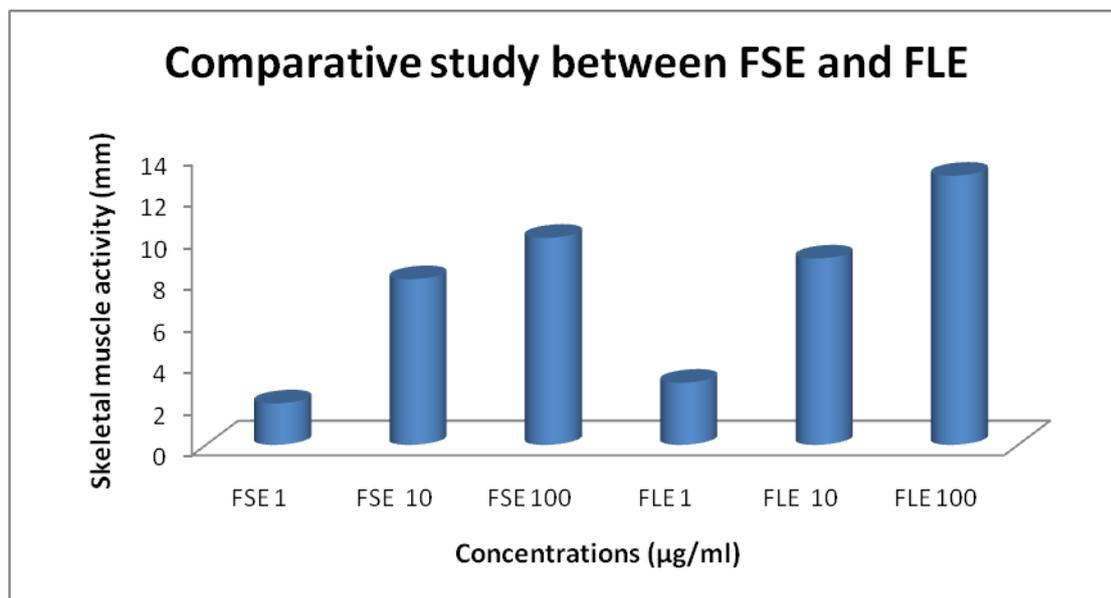


Fig 12: Comparative study between FSE and FLE

FSE1- Fenugreek Seeds Extract 1 $\mu\text{g/ml}$, FSE10- Fenugreek Seeds Extract 10 $\mu\text{g/ml}$, FSE100- Fenugreek Seeds Extract 100 $\mu\text{g/ml}$

FLE1- Fenugreek Leaves Extract 1 $\mu\text{g/ml}$, FLE10- Fenugreek Leaves Seeds Extract 10 $\mu\text{g/ml}$, FLE100- Fenugreek Leaves Extract 100 $\mu\text{g/ml}$.

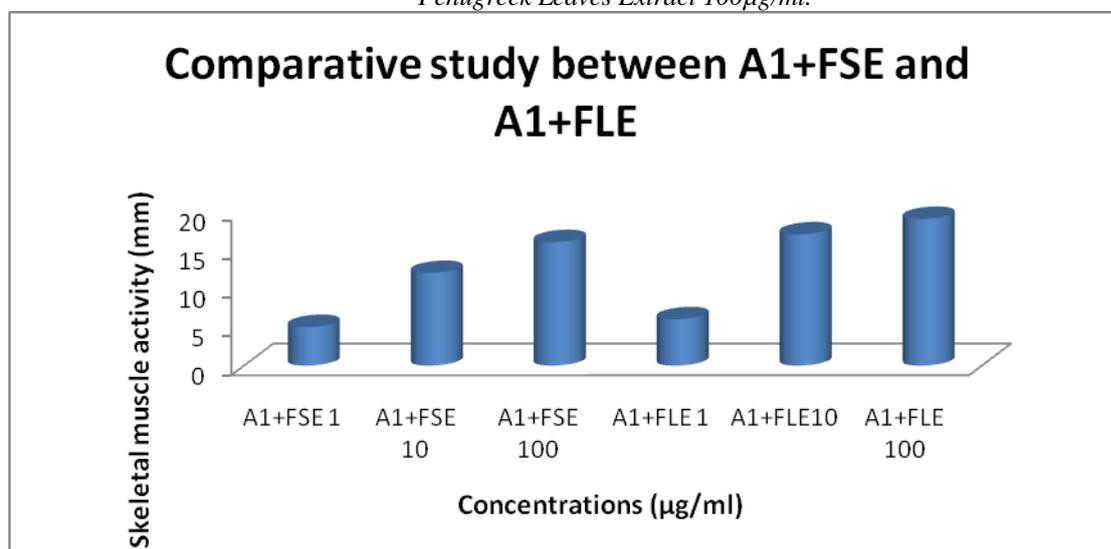


Fig 13: Comparative study between A1+FSE and A1+FLE

A1- Acetyl choline-1 $\mu\text{g/ml}$, FSE1- Fenugreek Seeds Extract 1 $\mu\text{g/ml}$, FSE10- Fenugreek Seeds Extract 10 $\mu\text{g/ml}$, FSE100- Fenugreek Seeds Extract 100 $\mu\text{g/ml}$, FLE1- Fenugreek Leaves Extract 1 $\mu\text{g/ml}$, FLE10- Fenugreek Leaves Seeds Extract 10 $\mu\text{g/ml}$, FLE100- Fenugreek Leaves Extract 100 $\mu\text{g/ml}$.

DISCUSSION:

The fenugreek seeds extract and fenugreek leaves extract was found to have skeletal muscle activity with the concentrations of 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, and 100 $\mu\text{g/ml}$.

When the activity was compared between the standard drug i.e, Acetylcholine and test drugs fenugreek seeds extract and fenugreek leaves 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 1 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 3 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$ and 8 $\mu\text{g/ml}$ and with d-tubocuraine of dose about 16 $\mu\text{g/ml}$. The acetylcholine were shown more activity by increasing the dose response whereas, the drug d-tubocuraine 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 fenugreek seeds extract of using different doses like 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$. For

both the tests drugs the response have been increased.

The effect of acetylcholine and fenugreek seeds extract (FSE) were compared and the result shown the more active response with the acetylcholine rather than the seeds extract

The effect of acetylcholine and fenugreek leaves extract were compared and the result shown the more active response with the acetylcholine rather than the fenugreek leaves extract

Then the effect of single seeds extract and combination of fenugreek seeds extract +acetylcholine is compared and the result shown more active with the combination of FSE+ACH.

Then the effect of single fenugreek leaves extract and combination of fenugreek leaves extract + Acetylcholine is compared and the result shown more active with the combination of FLC+ACH

But from both the combination ACH+FSE and ACH+FLE, the better and more active result was shown by the FLE+ACH.

The comparison study between FSE and FLE, the FLE have shown more active response than the FSE

Comparative study between A1+FSE and A1+FLE were studied and the result that the acetylcholine i.e, 1 µg/ml+FSE1 1µg/ml was less active than acetylcholine 1 µg/ml+FSE10 10 µg/ml and this is less active than acetylcholine 1 µg/ml+FSE100 100 µg/ml same result is also shown by the FLE by increasing the concentration, the active response as increased.

From both the comparison the maximum active response were shown by the ACH+FLE than ACH+FSE.

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

Thus, the present investigation proves that fenugreek seeds extract and fenugreek leaves extract were have good skeletal muscle activity alone and combination with acetylcholine and it produces the significant skeletal muscle activity at high concentration .

CONCLUSION:

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

Then skeletal muscle activity is evaluated by using test drugs fenugreek seeds extract of using different

doses like 1 µg/ml, 10 µg/ml and 100 µg/ml. For both the tests drugs the response have been increased. The effect of acetylcholine and fenugreek seeds extract (FSE) were compared and the result shown the more active response with the acetylcholine rather than the seeds extract

The effect of acetylcholine and fenugreek leaves extract were compared and the result shown the more active response with the acetylcholine rather than the fenugreek leaves extract.

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

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