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EVALUATION OF SKELETAL MUSCLE ACTIVITY OF *AZADIRACHTA INDICA* LEAVES AND FLOWERS EXTRACT ON FROGS RECTUS ABDOMINUS MUSCLE

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ABSTRACT
Azadirachta indica, commonly known as neem, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae. The skeletal muscle activity is evaluated by using test drugs neem 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 neem leaves extract (NLE)were compared and the result show the more active response with the acetylcholine rather than neem leaves extract the effect of acetylcholine and neem flowers extract were compared and the results shown the more active response with the acetylcholine rather than neem flowers extract.

KEYWORDS: *Azadirachta indica, skeletal muscle activity, neem leaves extract, neem flowers extract, acetylcholine*.

INTRODUCTION
AZADIRACHTA INDICA
Nomenclature
Azadirachta indica, commonly known as neem, nimtree or Indian lilac,[1,2] is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to the Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically grown in tropical and semi-tropical regions. Neem trees also grow in islands located in the southern part of Iran. Its fruits and flowers are the source of neem oil.

Description
Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most of its leaves or nearly all leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–25 metres (66–82 ft). The neem tree is very similar in appearance to its relative, the Chinaberry (*Melia azedarach*).

Leaves
The opposite, pinnate leaves are 20–40 centimetres (7.9–15.7 in) long, with 20 to 31 medium to dark green leaflets about 3–8 centimetres (1.2–3.1 in) long. The terminal leaflet often is missing. The petioles are short.

Flowers
The (white and fragrant) flowers are arranged in more-or-less drooping axillary panicles which are up to 25 centimetres (9.8 in) long. The inflorescences, which branch up to the third degree, bear from 250 to 300 flowers. An individual flower is 5–6 millimetres (0.20–0.24 in) long and 8–11 millimetres (0.31–0.43 in) wide. Protandrous, bisexual flowers and male flowers exist on the same individual tree.

Fruits
The fruit is a smooth (glabrous), olive-like drupe which varies in shape from elongate oval to nearly roundish, and when ripe is 1.4–2.8 centimetres (0.55–1.10 in) by 1.0–1.5 centimetres (0.39–0.59 in). The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3–0.5 centimetres (0.12–0.20 in) thick. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two, or three, elongated flowers (kernels) having a brown flower coat.

Cultivation
The neem tree is noted for its drought resistance. Normally it thrives in areas with sub-arid to sub-humid conditions, with an annual rainfall of 400–1,200 millimetres (16–47 in). It can grow in regions with an annual rainfall below 400 mm, but in such cases it depends largely on ground water levels. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils. It is a typical tropical to

subtropical tree and exists at annual mean temperatures of 21–32 °C (70–90 °F). It can tolerate high to very high temperatures and does not tolerate temperature below 4 °C (39 °F). Neem is one of a very few shade-giving trees that thrive in drought-prone areas e.g. the dry coastal, southern districts of India, and Pakistan. The trees are not at all delicate about water quality and thrive on the merest trickle of water, whatever the quality. In India and tropical countries where the Indian diaspora has reached, it is very common to see neem trees used for shade lining streets, around temples, schools and other such public buildings or in most people's back yards. In very dry areas the trees are planted on large tracts of land.

Scientific classification

Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A.indica

Uses

Products made from neem trees have been used in India for over two millennia for their medicinal properties. Neem products are believed by Siddha and Ayurvedic practitioners to be anthelmintic, antifungal, antidiabetic, antibacterial, an tiviral, contraceptive, and sedative. It is considered a major component in siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. Neem oil is also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels. Neem leaves have also been used to treat skin diseases like eczema, psoriasis, etc.

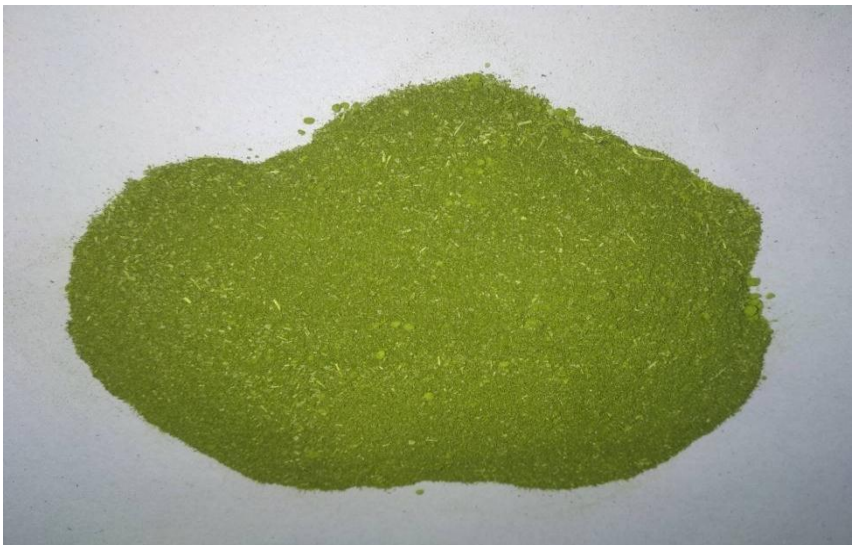
Insufficient research has been done to assess the purported benefits of neem, however. In adults, short-term use of neem is safe, while long-term use may harm the kidneys or liver; in small children, neem oil is toxic and can lead to death. Neem may also cause miscarriages, infertility, and low blood sugar.

COLLECTION OF PLANT MATERIAL

A) Neem *(Azadirachta indica)* leaves were collected from the botanical garden of vaageswari institute of pharmaceutical science, Karimnagar, telengana.

PREPARATION OF *AZADIRACHATA INDICA* LEAVES EXTRACT

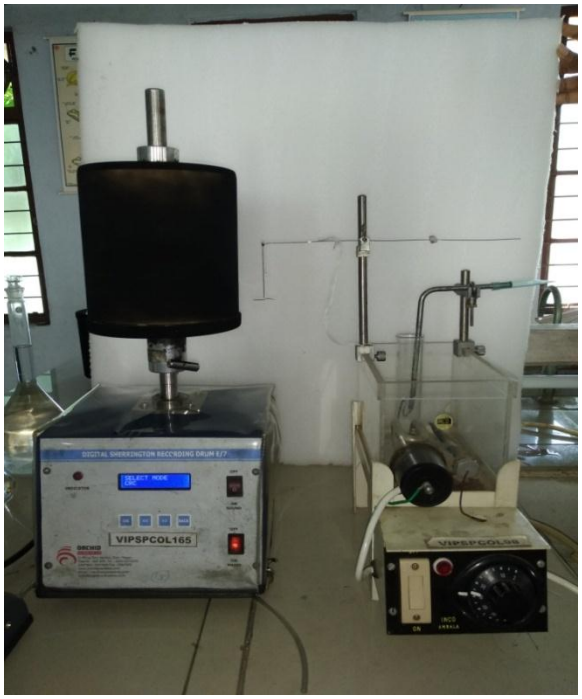
50 grams of neem leaves were obtained and washed. The collected leaves are dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh. Soxhalation with water (aqueous) for 10-20 cycles. The final product was dried and weighed.



EFFECT OF *AZADIRACHATA INDICA* LEAF EXTRACT ON THE SKELETAL MUSCLE OF THE FROG

Since the animigrane drugs were reported to have skeletal muscle activity, so this experiment was attempted to assess the effect of *AZADIRACHATA INDICA* leaves extract on the frogs rectus abdominus muscle preparation. The experiment was carried as per method described by KULAKARNI.

Frogs weighing 20-25 grams were used in this study. The frog was stunned and decapitated and 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 as its base and the pair of muscls attached to it were dissected and trasfered to a dish containing frogs ringer solution at room temperature. The muscles were then carefully cleaned and one of them was trimmed to the desired size and mount in and organ bath filled with ringers solution at room temperature and aerated by stream of fine bubbles emerging near the buttom of the bath. Isotonic contractions were recorded using gimbel lever with a sideways writing point. The lever was balanced for a tension of approximately 2-5 grams. An extra load od approximately 1gm on the long arm was supplied because sometime the lever may not return to the baseline after washing. The drug period allowed for stabilization was 30min during which the muscle was subjected to 1gm stretch. At 0^{th} min –the kymograph was started after rising the extra load; in the 1^{st} min-the drug was added and in the 2^{nd} min-thekymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5^{th} min-the lever point was brought to the baseline and the cycle was started. After recording the graded the responses to different long dose of Ach, the *AZADIRACHATA INDICA* leaves extract was added and their was upon Ach induced contraction as well as the effect of its own in the tissue was studied.



B) Neem *(Azadirachata indica)* flowers were collected from the botanical garden of Vaageswari institute of pharmaceutical sciences, karimnagar, Telengana.

PRPARATION OF NEEM FLOWER EXTRACT

50grams of neem flowers were obtained and washed. The collected flowers were dried at room temperature, pulvarised by mechancical griender, sieved through mesh. Soxhalation with water (aqueous) for 20-30 cycles. The final product was dried and weighed.

EFFECT OF NEEM FLOWER EXTRACT (NFE) ON THE SKELETAL MUSCLE OF THE FROG

Since the animigrane drugs were reported to have skeletal muscle activity, so this experiment was attempted to assess the effect of *AZADIRACHATA INDICA* leaves extract on the frog rectus abdominus muscle preparation. The experiment was carried as per method described by KULAKARNI.

Frogs weighing 20-25 grams were used in this study. The frog was stunned and decapitated and 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 as its base and the pair of muscls attached to it were dissected and trasfered to a dish containing frogs

ringer solution at room temperature. The muscles were then carefully cleaned and one of them was trimmed to the desired size and mount in and organ bath filled with ringers solution at room temperature and aerated by stream of fine bubbles emerging near the buttom of the bath. Isotonic contractions were recorded using gimbel lever with a sideways writing point. The lever was balanced for a tension of approximately 2-5 grams. An extra load od approximately 1gm on the long arm was supplied because sometime the lever may not return to the baseline after washing. The drug period allowed for stabilization was 30min during which the muscle was subjected to 1gm stretch. At 0^{th} min –the kymograph was started after rising the extra load; in the 1^{st} min-the drug was added and in the 2^{nd} min-thekymograph was stopped. The tissue was washed and allowed to relax by applying an extra load. At the 5^{th} min-the lever point was brought to the baseline and the cycle was started. After recording the graded the responses to different long dose of Ach, the *AZADIRACHATA INDICA* leaves extract was added and their was upon Ach induced contraction as well as the effect of its own in the tissue was studied.

RESULTS

Skeletal muscle activity of Acetylcholine, d-tubocurarine, NLE, NFE, Acetylcholine+ NLE and Acetylcholine+ NFE.

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	NLE	1	2	Increased
8	NLE	10	5	Increased
9	NLE	100	9	Increased
10	NFE	1	3	Increased
11	NFE	10	8	Increased
12	NFE	100	11	Increased
13	Acetylcholine+ NLE	1 1	3	Increased
14	Acetylcholine+ NLE	1 10	6	Increased
15	Acetylcholine+ NLE	1 100	10	Increased
16	Acetylcholine+ NFE	1 1	4	Increased
17	Acetylcholine+ NFE	1 10	10	Increased
18	Acetylcholine+ NFE	1 100	13	Increased

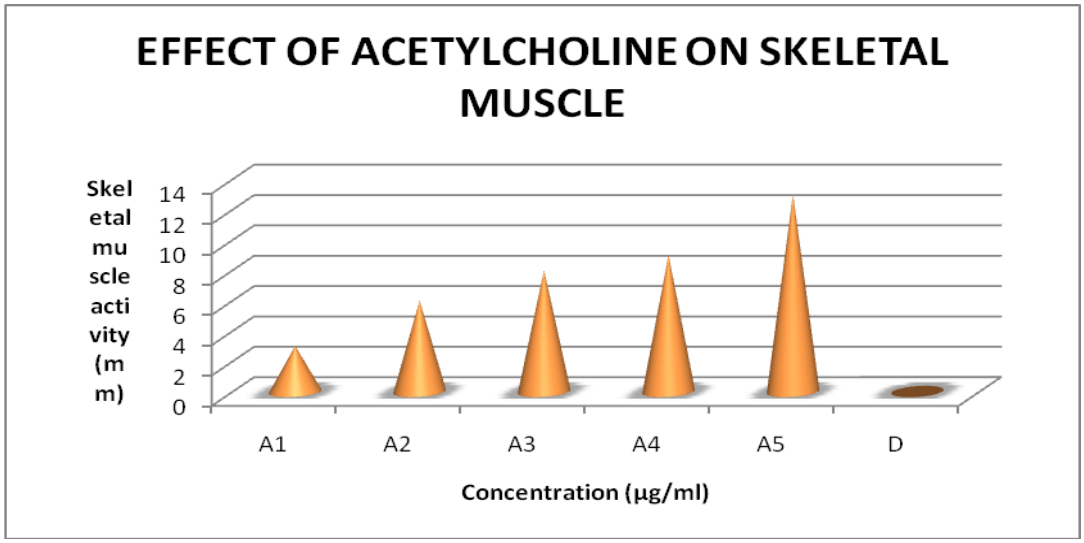


Figure No. 1: EFFECT OF ACETYLCHOLINE ON SKELETAL MUSCLE.

A1-Acetylcholine (1µg/ml), A2-Acetylcholine (2µg/ml), A3- Acetylcholine (µg/ml), A4-(8µg/ml), A5-Acetylcholine (16µg/ml) and d-tubocurarine(4µg/ml).

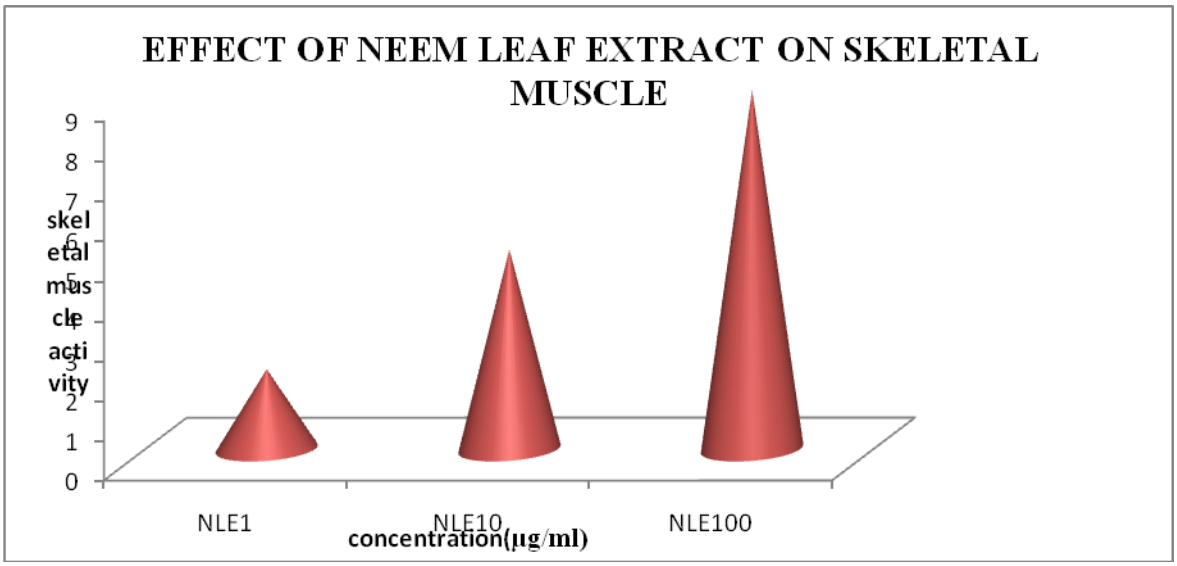


Figure No. 2: EFFECT OF NLE ON SKELETAL MUSCLE.

NLE1- Neem leaf extract (1µ/ml), NLE10 (10µg/ml), NLE100 (100µg/ml).

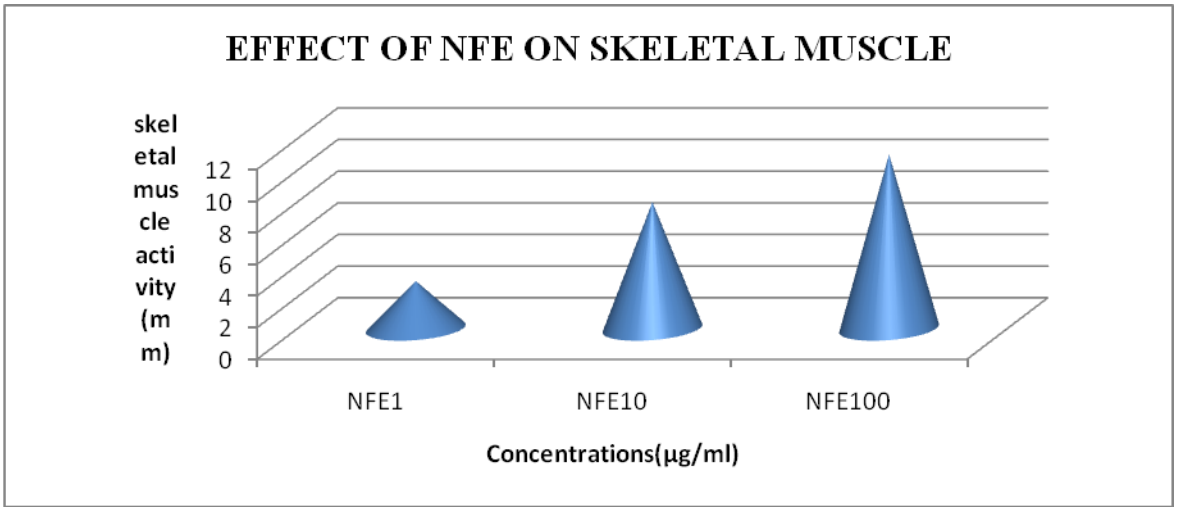


Figure No. 3: EFFECT OF NFE ON SKELETAL MUSCLE.

NFE1-Neem flower extract (1µg/ml), NFE10-(10µg/ml), NFE100-(100µg/ml).

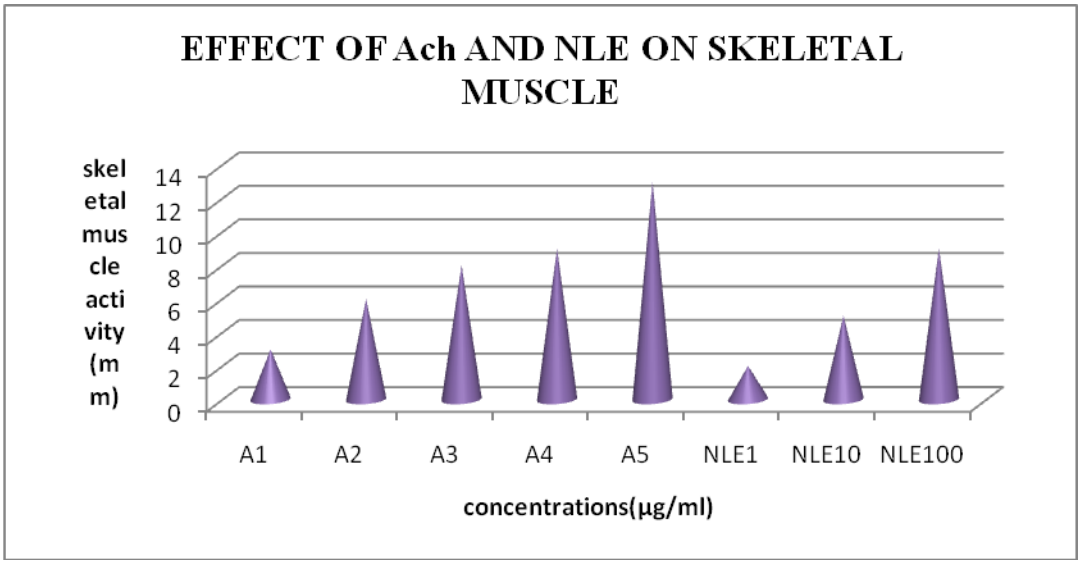


Figure No. 4: EFFECT OF Ach AND NLE ON SKELETAL MUSCLE.

A1-Acetylcholine(1µg/ml), A2(2µg/ml), A3(4µg/ml), A4(8µg/ml), A5(16µg/ml), NLE1-Neem leaf extract(1µg/ml), NLE10(10µg/ml), NLE100(100µg/ml).

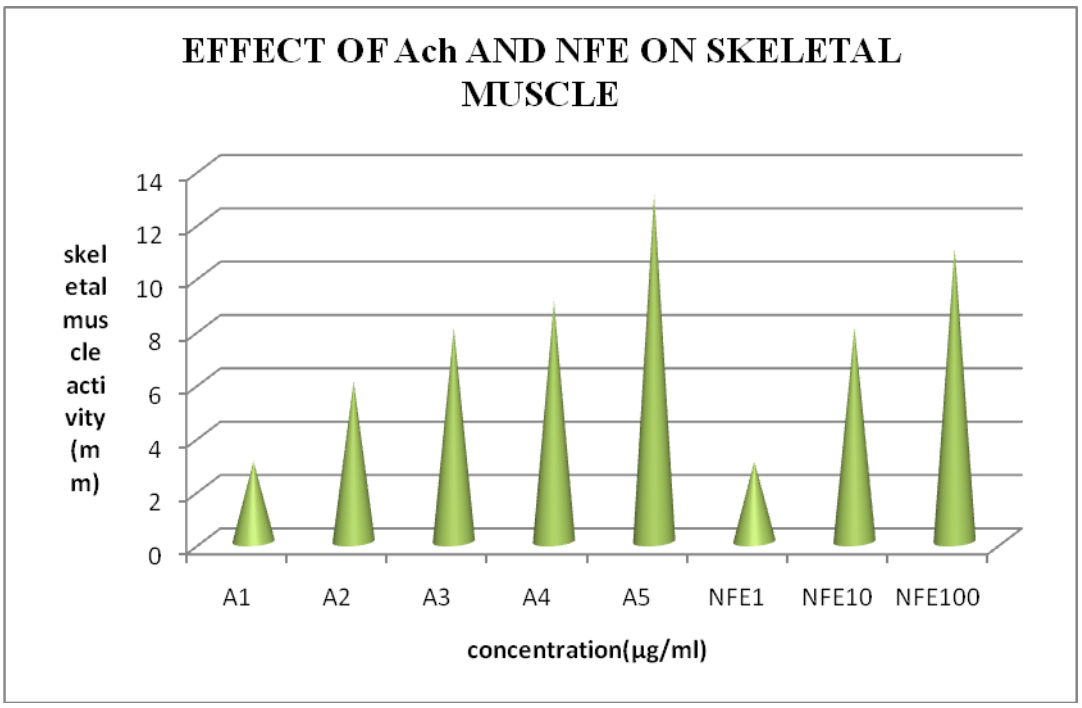


Figure No. 5: EFFECT OF Ach AND NFE ON SKELETAL MUSCLE.

A1-Acetylcholine(1µ/ml), A2(2µg/ml), A3(4µg/ml), A4(8µg/ml), A5(16µg/ml), NFE1Neem flower extract (1µg/ml), NFE10(10µg/ml), NFE100(100µg/ml).

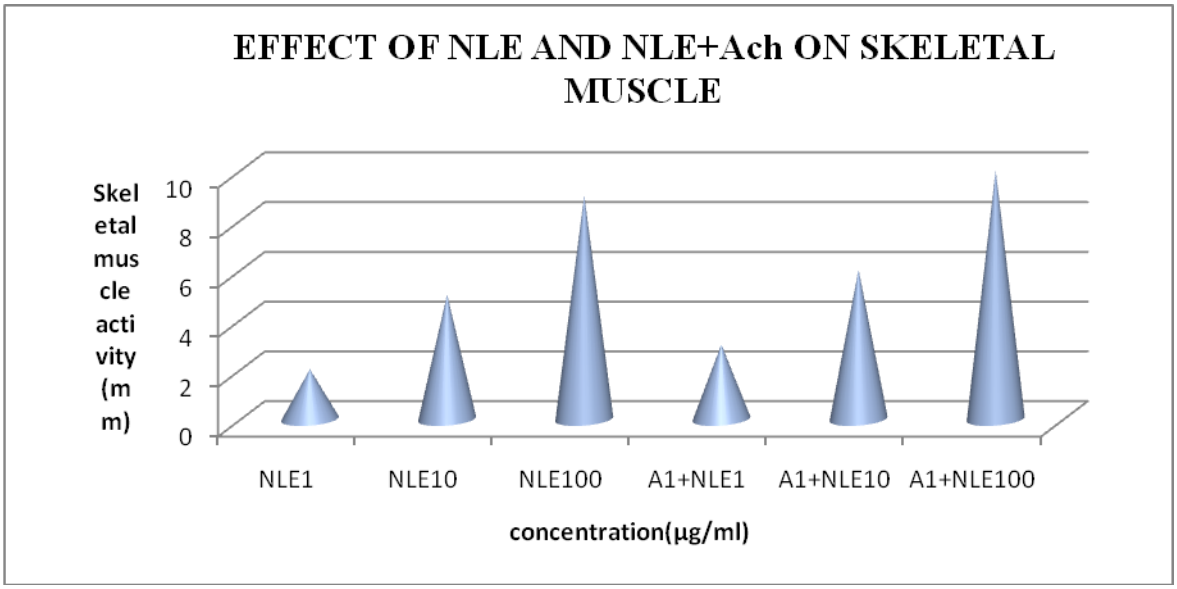


Figure No.6: EFFECT OF Ach AND NLE ON SKELETAL MUSCLE.

A1-Acetylcholine(1µg/ml), NLE1(1µg/ml), NLE10(10µg/ml), NLE100(100µg/ml).

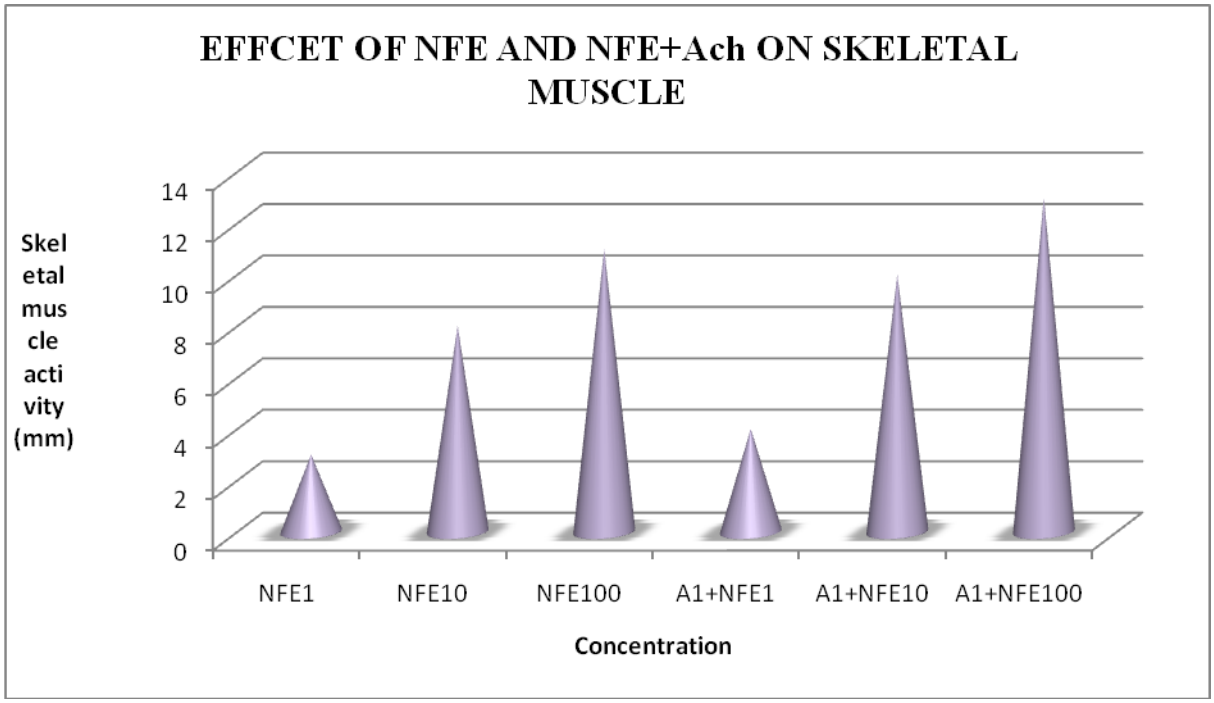


Figure No. 7: EFFECT OF Ach AND NFE ON SKELETAL MUSCLE.

A1-Acetylcholine (1µg/ml), NFE1(1µg/ml), NFE10(10µg/ml), NFE100(100µg/ml).

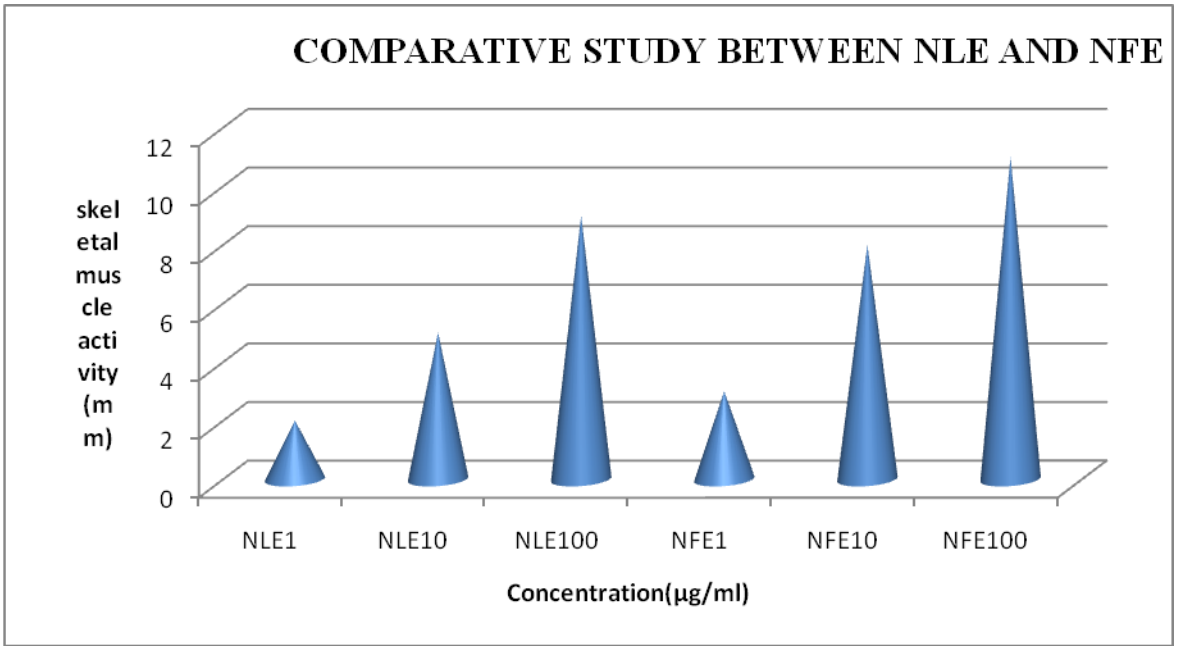


Figure No. 8: COMPARATIVE STUDY BETWEEN NLE AND NFE.

NLE1(1µg/ml), NLE10(10µg/ml), NLE100(100µg/ml), NFE1(1µg/ml), NFE10(10µg/ml), NFE100(100µg/ml).

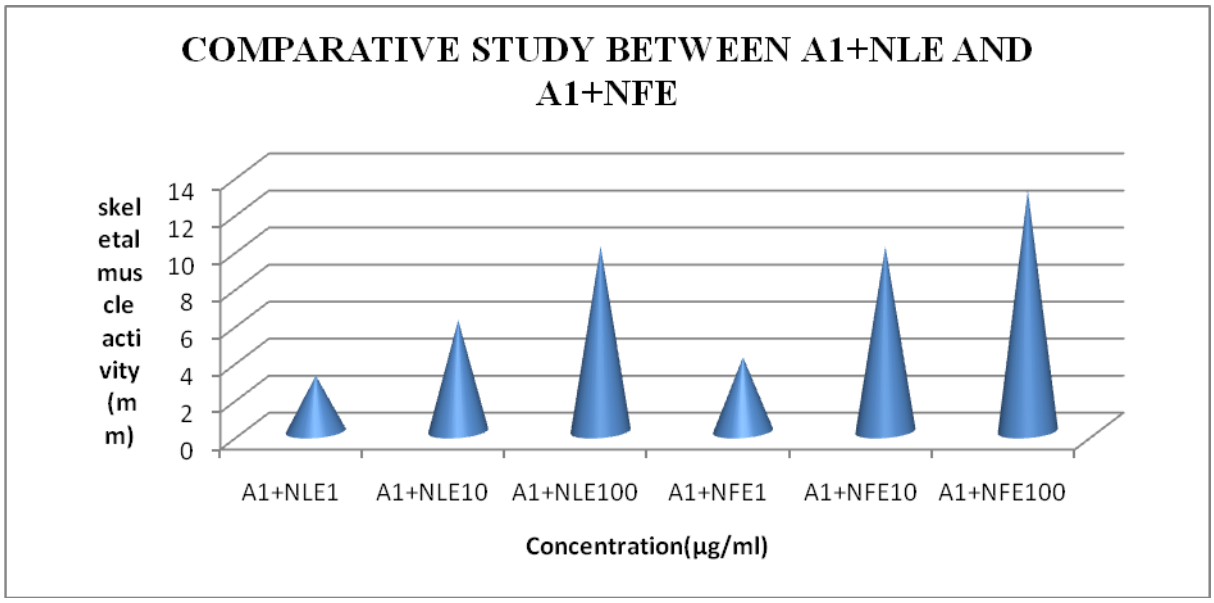
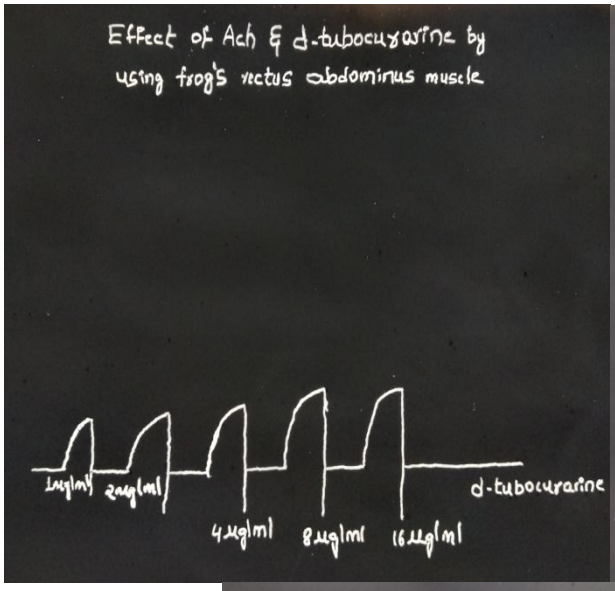
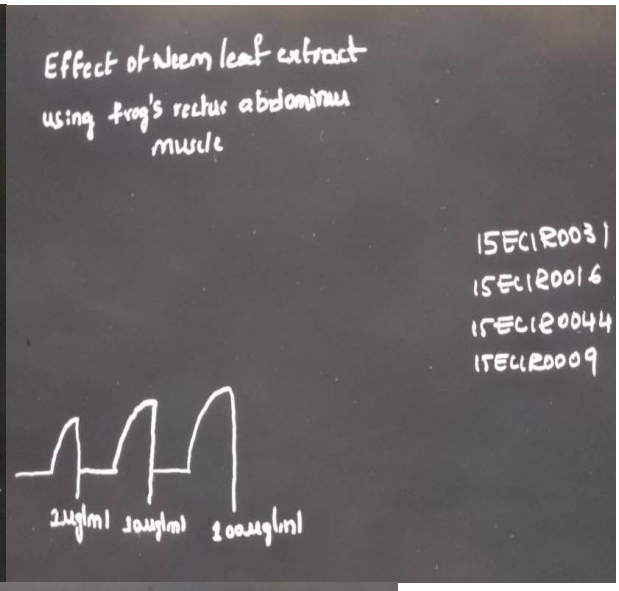
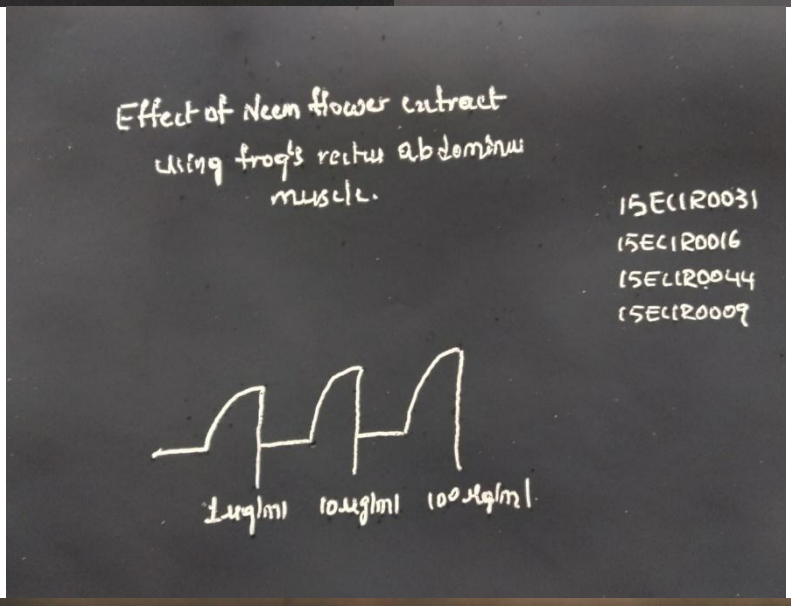


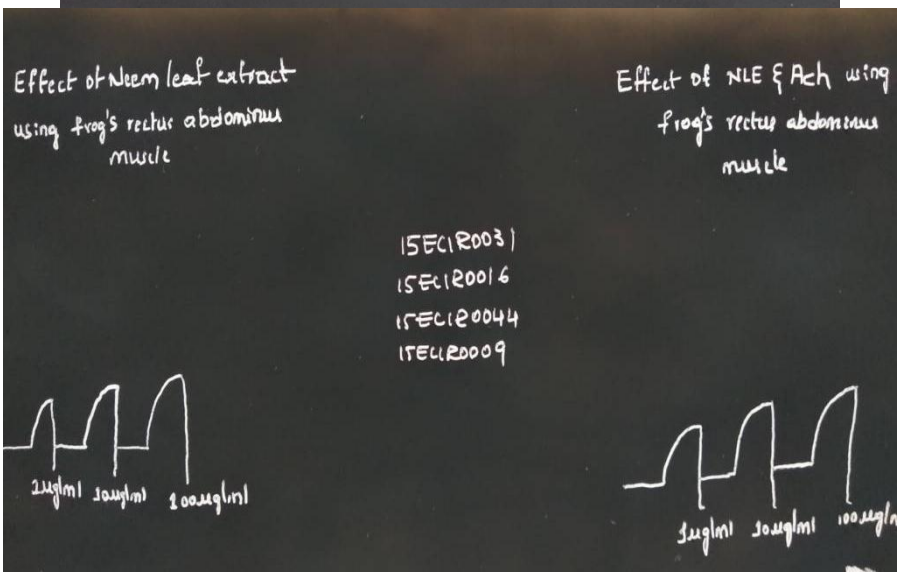
Figure No. 9: COMPARATVIVE STUDY BETWEEN A1+NLE AND A1+NFE.

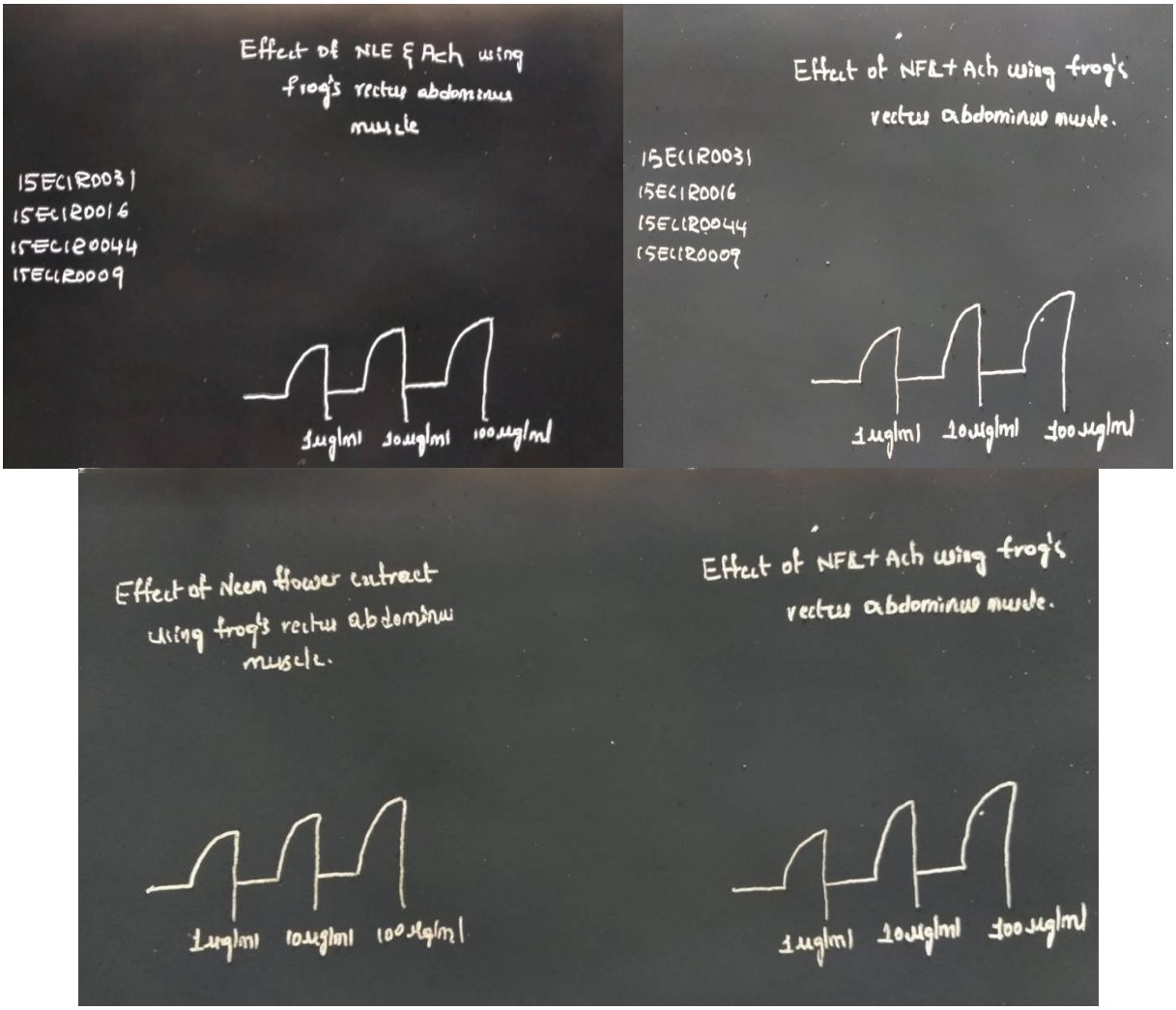
A1-Acetylcholine(1µg/ml),NLE1(1µg/ml), NLE10(µg/ml), NLE100(µg/ml), NFE1(1µg/ml), NFE10(10µg/ml), NFE100(100µg/ml).


Effect of Ach & d-tubocurarine by using frog's rectus abdominus muscle
1ug/ml
2ug/ml
4ug/ml
8ug/ml
16ug/ml
d-tubocurarine


Effect of Neem leaf extract using frog's rectus abdominus muscle
15EC1R0031
15EC1R0016
15EC1R0044
15EC1R0009
2ug/ml
10ug/ml
100ug/ml


Effect of Neem flower extract using frog's rectus abdominus muscle.
15EC1R0031
15EC1R0016
15EC1R0044
15EC1R0009
1ug/ml
10ug/ml
100ug/ml


Effect of Neem leaf extract using frog's rectus abdominus muscle
15EC1R0031
15EC1R0016
15EC1R0044
15EC1R0009
2ug/ml
10ug/ml
100ug/ml
Effect of NLE & Ach using frog's rectus abdominus muscle
1ug/ml
10ug/ml



DISCUSSION

The neem 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 neem 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 neem leaves extract and neem flowers 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 NLE and were compared and the result shown the more active response with the acetylcholine rather than the NFE.

The effect of single TSE and combination of NFE +ACH, and effect of single NLE and combination of NLE+ACH is compared and the result shown more active with the combination.

But from both the combination ACH +NFE and ACH +NLE, the better and more active results was shown by the NLE+ACH.

The comparision study between NFE and NLE, the TLE have shown more active response than NFE.

Comparative study between A1+NFE and A1+NLE were studied and the result that the acetylcholine i.e., 1µg/ml +NFE1 1µg/ml was less active than acetylcholine 1µg/ml+NFE5 10µg/ml and this is less than acetylcholine 1µg/ml+NFE100 100µg/ml same results is also shown by the NLE by increasing the concentration, the active response is increased.

From both the comparision the maximum active response were shown by the ACH+NLE than ACH +NFE.

Thus from present study it was concluded that the Neem leaves extract has good skeletal muscle activity than the Neem flowers extract.

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

SUMMARY

The Neem leaves aqueous extract and Neem flowers aqueous extract was found to have skeletal muscle activity with the concentration of 1µg/ml, 10µg/ml, and 100µg/ml.

When the activity was compared between the standard drug i.e, Acetylcholine and test drug Neem leaves extract and Neem flowers 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 dose about 4µg/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.

The skeletal muscle activity is evaluated by using test drug neem leaves extract of using different doses like 1µg/ml, 10µg/ml, 100µg/ml. For both the tests drugs the response has been increased.

The effect of acetylcholine and neem flowers extract were compared and the results shown the more active responses with the acetylcholine rather than the neem flowers extract.

The effect of single neem leaves extract and combination of hibiscus extract+acetylcholine is compared and the result shown more active with the combination of NLE+ACH.

But from the combination ACH+NLE and ACH+NFE, better and more active result was shown by the ACH+NLE.

The comparision study between NLE and NFE, the NLE have shown more active response than the NFE.

Comparative study between A1+NLE and A1+NFE were studied and the result that the acetylcholine i.e, 1µg/ml+NLE 1µg/ml was less active than acetylcholine 1µg/ml and this is less active than acetylcholine 1µg/ml+NLE100µg/ml same result is also shown by the NFE by increasing the concentration, the active response as increased.

From the both comparision the maximum active response were shown by the ACH+NLE than ACH+NFE.

Thus from the present study it was concluded that Neem leaves extract was good skeletal activity than the Neem flowers extract.

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

CONCLUSION

The Neem leaves and Neem flowers 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 neem leaves extract and neem flowers 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 neem 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 neem leaves extract (NLE) were compared and the result show the more active response with the acetylcholine rather than neem leaves extract the effect of acetylcholine and neem flowers extract were compared and the results shown the more active response with the acetylcholine rather than neem flowers extract.

The study finally concluded that the effect of neem leaves extract and combination of neem leaves extract and acetylcholine is compared and the results shown more active with the combination of neem flowers 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.

REFERENCES

1. Verkerk RHJ, Wright DJ. Activity of neem seed kernel extract and synthetic azadirachtin against larvae of Plutella xylostellal. Pesticide science, 1993; 37: 83-91.
2. Mitra, C. R., Neem, Dr M. S. Patel, Indian Central Oilseeds Committee, Hyderabad, 1963; 69–94.
3. N Jamra, G Das, P Singh, M Haque. Anthelmintic efficacy of crude neem (Azadirachta indica) leaf powder against bovine strongylosis. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology, 2015; 39(4): 786-788.

4. Joshi S.N., Dutta S., Kumar B.K., Katti U., Kulkarni S., Risbud A., Mehendale S., Expanded safety study of Praneem polyherbal vaginal tablet among HIV-uninfected women in Pune, India: a phase II clinical trial report, 2008; 84(5): 343-347.
5. Menakshi Bhat, Sandeepkumar K.K., Tirmale A.R., Bhargava S.Y., Joshi BN. Antidiabetic Properties of Azardiracta indica and Bougainvillea spectabilis: In Vivo Studies in Murine Diabetes Model. Evid Based Complement Alternat Med., 2009; 23: 1-8.
6. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta P, Sarkar D. Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer. Life. Sci, 2004; 75: 2867-2878.
7. Ashis KM, Robin D, Debashree S (2008) Isolation of a snake venom phospholipase A2 (PLA2) inhibitor (AIPLAI) from leaves of Azadirachta indica (Neem): Mechanism of PLA2 inhibition by AIPLAI in vitro condition. Toxicon, 51(8): 1548-1553.
8. Shravan Kumar, D. Ruqsar, K. Malathi, S. Akhila, P. Abhilash, P. (2015). Evaluation of skeletal muscle activity of fenugreek seeds extract and fenugreek leaves extract on isolated frog's rectus abdominus muscle. Indo American journal of pharmaceutical sciences, 2015; 2(4): 763-770.
9. Anvesh Nag, P. Samatha, P. Rajitha, T. Susheel, R. Rekha, R. Rakesh, R. Shravan kumar, D. Ramakrishna, R. (2015). Evaluation of skeletal muscle activity of *Aloe vera* extract on frog's rectus abdominus muscle International journal of phytopharmacy research, 6(2): 61-64.
10. Shravan Kumar D, Rajashekar CH. Evaluation of skeletal muscle activity of *Hibiscus rosa sinesis* leavesextract on frog's rectus abdominus muscle, European journal of Biomediacal and Pharmaceutical sciences, 2019; 6(3): 473-478.