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EVALUATION OF SKELETAL MUSCLE ACTIVITY OF *HIBISCUS ROSA SINENSIS* LEAVES EXTRACT ON ISOLATED FROG'S RECTUS ABDOMINUS MUSCLE

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1. INTRODUCTION

Hibiscus Rosa-Sinensis: *Hibiscus rosa-sinensis,* known colloquially as Chinese hibiscus, China rose, Hawaiian hibiscus, and shoe black plant, is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family *Malvaceae*, native to East Asia.

Nomenclature: *Hibiscus rosa-sinensis* was named in 1753 by Carl Linnaeus in his Species Plantarum. The Latin term rosasinensis literally means "rose of China", though it is not closely related to the true roses.

Description: *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5m (8–16ft) tall and 1.5–3m (5–10ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. The 5-petaled flowers are 10cm (4in) in diameter, with prominent orange-tippedred anthers.

Vegetative characters

The root is a branched tap root. The stem is aerial, erect, green, cylindrical and branched. The leaf is simple, with alternate phyllotaxy and is petiolate. The leaf shape is ovate, the tip is acute and margin is serrated. Venation is unicostate reticulate. (Venation is branched or divergent.) Free lateral stipules are present.

Floral characters

The flower is complete (bisexual), actinomorphic, bracteate or ebracteate, bracteolate or ebracteolate, pedicellate, dichlamydeous, regular, pentamerous, hypogynous, and solitary. It can bloom all year round.

Cultivation

It is widely grown as an ornamental plant throughout the tropics and subtropics. As it does not tolerate temperatures below 10 °C (50 °F), in temperate regions it is best grown under glass. However, plants in containers may be placed outside during the summer months or moved into shelter during the winter months. Numerous varieties, cultivars, and hybrids are available, with flower colors ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals.

Image.1: *Hibiscus* Plant.

Scientific classification

Kingdom	**Plantae**
Division:	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Malvales
Family:	Malvaceae
Genus:	*Hibiscus*
Species:	*H. rosa-sinensis*

Image: Classification Of Hibiscus

Uses: The flowers of *Hibiscus rosa-sinensis* are edible and are used in salads in the Pacific Islands. The flower is additionally used in hair care as a preparation. It can also be used as a pH indicator. When used, the flower turns acidic solutions to a dark pink or magenta color and basic solutions to green. In several countries the flowers are dried to use in a everage, usually tea.

Hibiscus rosa-sinensis is considered to have a number of medical uses in Chinese herbology. It may have some potential in cosmetic skin care; for example, an extract from the flowers of *Hibiscus rosa-sinensis* has been shown to function as an anti-solar agent by absorbing ultraviolet radiation.

2. MATERIALS AND METHODOLOGY

Collection of plant Material: Hibiscus (*Hibiscus rosa sinenses*) leaves were collected from the botanical garden of vaageswari institute of pharmaceutical sciences, Karimnagar, Telangana.

Preparation of plant Extract: 30 gm of Hibiscus leaves were obtained and washed. The collected leaves were dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh was soxhalation with ethanol for 10-20 cycles. The final product was dried and weighed.[1-3]

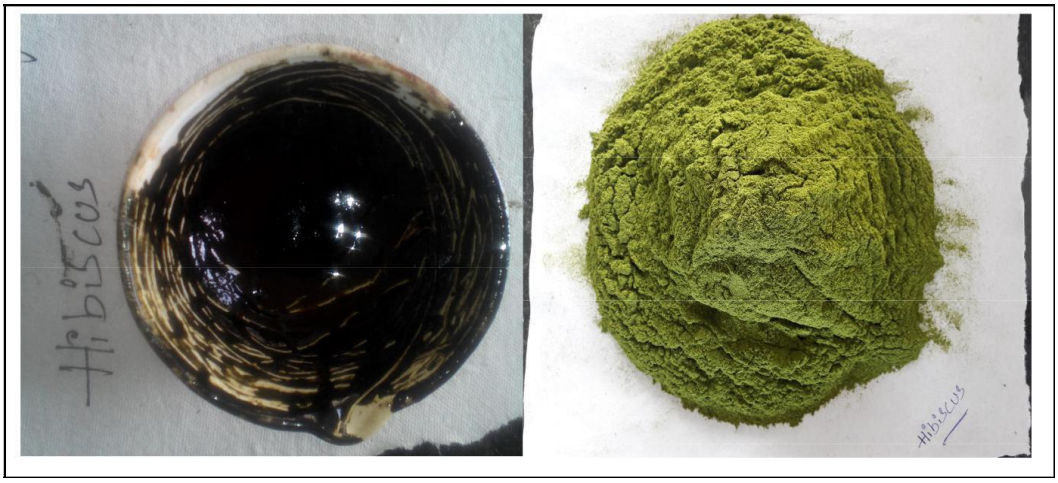


Image. 2: Hibiscus Extract & Leaves Powder.

Image.3: Sohxalation of Hibiscus

Image.4: Frog

Effect of Hibiscus Leaves Extract (HLE) on The Skeletal Muscle of The Frog

This experiment was attempted to assess the effect of Hibiscus leaves extracts on the frog rectus abdominis muscle preparation. The experiment was carried as per method described by Kulkarni (The text book of experimental pharmacology).

Frogs weighing 20-25 gm 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 muscle 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 2-5 gm. An extra load of approximately 1gm on the long arm was supplied because some time the lever may not return to the base line after washing .The drug period allowed for stabilization was 30 min during which the muscle was subjected to 1gm 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 long dose of acetylcholine, the Hibiscus leaves extract was added and their effect upon acetylcholine induced contraction as well as the effect of its own in the tissue was studied.[4-6]

3. RESULTS

Table 1: Skeletal muscle activity of Acetylcholine, d-tubocuraine, HLE, Acetylcholine+HLE.

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-tubocuraine	4	-	-
7	HLE	1	2	Increased
8	HLE	10	6	Increased
9	HLE	100	8	Increased
13	Acetylcholine + HLE	1 1	5	Increased
14	Acetylcholine + HLE	1 10	8	Increased
15	Acetylcholine + HLE	1 100	10	Increased

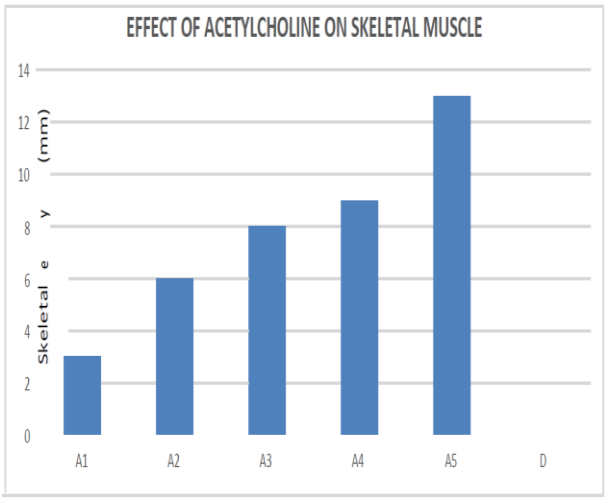


Figure No.1: Effect of Acetylcholine on Skeletal Muscle.

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

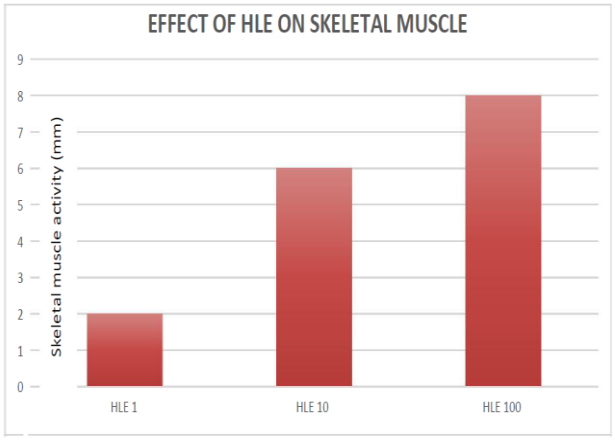
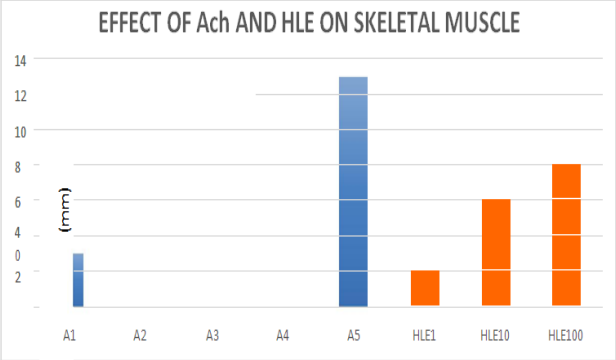


Figure No. 2: Effect of Hle on Skeletal Muscle.

HLE 1-Hibiscus Leaves Extracts (1µg/ml), HLE 10-Hibiscus Leaves Extracts (10µg/ml), HLE 100-Hibiscus Leaves Extracts (100µg/ml)



A1-Acetylcholine (1µg/ml), A2-Acetylcholine (2µg/ml), A3-Acetylcholine (4µg/ml), Acetylcholine (8µg/ml), A5-Acetylcholine (16µg/ml), HLE 1-Hibiscus Leaves Extracts (1µg/ml), HLE 10-Hibiscus Leaves Extracts (10µg/ml),HLE 100-Hibiscus Leaves Extracts (100µg/ml)

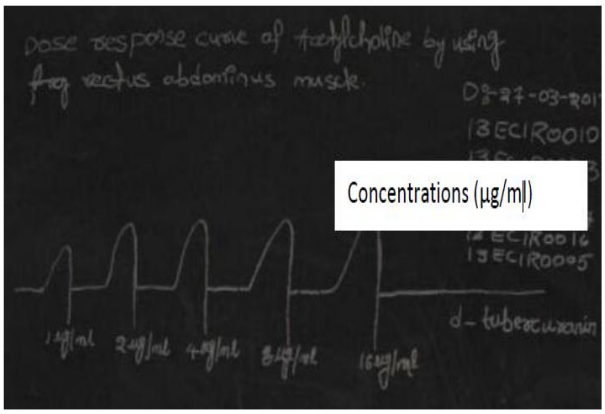


Image No. 5: kymograph- Effect of Ach on skeletal muscle.

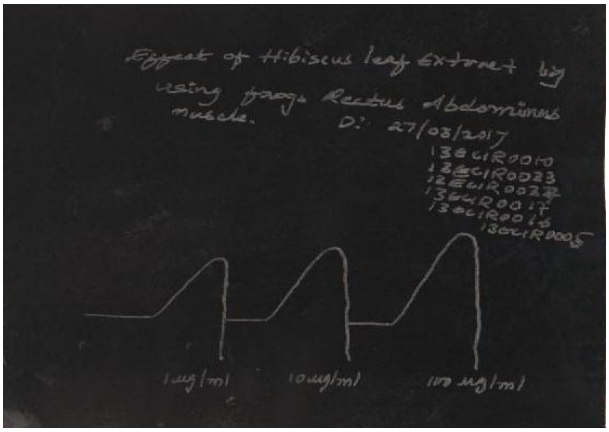


Image No.6: Kymograph- Effect of HLE.

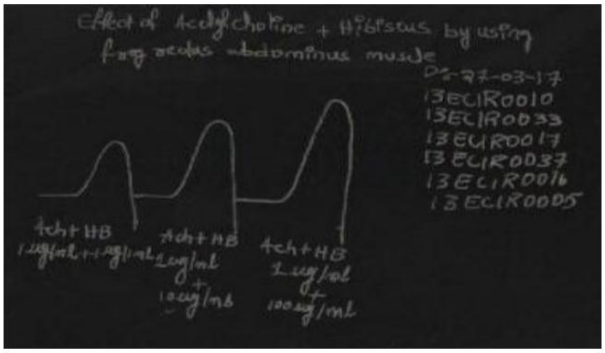


Image No. 7: Kymograph- Effect of Ach + HLE on the skeletal muscle.

4. DISCUSSION

SKELETAL MUSCLE has a number of important functions. Skeletal muscle maintains posture, produce locomotion, and in the case of the case of the respiratory muscle, ventilate the lungs. In addition, the skeletal muscle makes a major contribution to metabolic control by taking up glucose (storing it as glycogen) and oxidizing fatty acids to blood glucose and lipid levels.

The basic unit of a skeletal muscle is a muscle fiber, a single multi nucleated cell extending along the length of the muscle. There are different types of muscle fibers adopted for different purposes.

Achondroplasia: It is an autosomal dominant disorder that affects growth and development of long bones. It is characterized by bone deformation, leading to disproportionate shortness of the extremities (legs, arms, fingers and toes) relative to the trunk.

Achondrogenesis: It includes a set of disorders resulting due to growth hormone deficiency, which leads to altered bone and cartilage development. The infants are stillborn or shortly after birth.

Clubfoot: Also known as *talipes equinovarus*, it is the most common congenitial disorder which affects joints of the feet. In babies born with this disorder, one foot or both the feet point downwards and inwards, making it difficult for them to walk and move around.

Hereditary Multiple Exostoses: Inherited in an autosomal dominant pattern, this disorder involves the development of benign (non-cancerous) bone tumors called exostoses. Such tumors cause uneven limb growth and limited joint movements.

Osteogenesis Imperfecta: Also known as *brittle bone disease*, this genetic anomaly leads to underproduction of cartilage causing fragile bones, loose joints, and blue sclerae. It is also inheritedin an autosomal dominant pattern. Osteopetrosis: It is a rare inherited disorder where in bone reabsorption is altered due to dysfunctional osteoclasts. This leads to increased bone density and hardening of bones.

Spina Bifida: It is neural tube defect in which the skeletal tissues surrounding the spinal cord remain underdeveloped. Under normal conditions, the two sides of each vertebra join together to form a column around the spinal cord. In case of this disorder, such fusion does not take place in certain vertebrae, leaving a slit in the spinal column.

Fibrous Dysplasia: It is a gene mutation, which is it hereditary and occurs during the fetal development in the mother's womb. Fibrous dysplasia is a condition where fibrous tissues start growing in place of normal bones. The fibrous tissues tissue expands during the growth of the bones making them weaker and weaker. If the mutation occurs at the early stage of fetal development it affects more tissues however, if it occurs late a very few get affected. Since mutation occurs before birth it is considered as a genetic disorders but it is not hereditary because the sperm or egg does not pass on the mutation to the fetus.

Hypohosphatemia: The decrease in phosphorous levels I the body results in hypophosphatemia. Conditions like chronic diarrhea, starvation, alcoholism, vitamin D deficiency, etc., can lead to this conditions. The person suffering from hypophosphatemia will experience muscle weakness and pain in the bones. Adults suffering from this condition also experience loss of teeth at an early age and the bones become susceptible to fractures. Children with hypophosphatemia have an abnormally shaped head and their limbs are extremely short with enlarged joints.

Arthritis: It is a group of diseases involving inflammation of joints. Autoimmuntiy, wear and tear of the joints and associated tissues, ad infection are the common causes. Depending on the cause and the tissue affected, about 100 different types have been identified. Osteoarthritis, rheumatoid arthritis and psoriatic arthritis are some of the common types.

Bursitis: It implies the inflammation of fluid-filled sacs called *bursae*, which serve as a cushion between the bone, and the tendons and/or muscles present in joints. Shoulders, knees, elbows and hips are the commonly affected joints. Such inflammation can occur due to infection, trauma, oraging.

Osteomyelitis: It is the result of bone infection, either contracted through another infected organ of the body or body or after surgeries involving metal plates and rods. It is characterized by severe pain and inflammation in the infected bone, fever, chills, nausea, and weakness. *Staphylococcus aureus* is the most common causative agent of osteomyelitis. Osteoporosis: It is one of the most common bone diseases, and is characterized by reduction I bone mineral density. It is the result of an imbalance in the bone formation and bone re-absorption process. The bones become weak and tend to get fractured easily.

Rickets: This disorder involves the softening and weakening of bones due to the deficiency of vitamin D, calcium and phosphate. The symptoms include pain and tenderness of bones, muscle cramps, dental and skeletal deformities, etc.

Scoliosis: Scoliosis is a condition involving excessive or abnormal curvature of the spine. It may be idiopathic (spontaneously arising), congenital or neuromuscular. It is characterized by chronic backache ad lower back pain as well as difficulty I bending, twisting or lifting objects.

Kyphosis: This is also a condition of abnormal spine curvature, ad is characterized by formation of a hunchback. Infection, connective tissue disorders, degenerative spine diseases, and muscular dystrophy, are some of the causes of kyphosis.

Osteonecrosis: Insufficient blood supply to large joints such as hips, shoulders , elbows and knees leads to death of the bones tissues in that area . This is termed as osteonecrosis. This can occur due to injury, trauma, radiation therapy, fractures, and bone dislocates.

Scurvy: It is the disease caused by the deficiency of vitamin C or ascorbic acid in the body. This deficiency leads to poor recovery of wounds and the person becomes more susceptible to bruises, it causes gum diseases, weakness and skin haemorrhages. Although an uncommon health condition, scurvy affects older and malnourished adults. The major cause of scurvy is over cooking of the food as it destroys the vitamin C content in the food.

Poliomyelitis: Also called polio, this diseases is highly contagious and infectious and is caused by three types of polio viruses. It affects the nervous system resulting into partial or total paralysis. It is often transmitted through fecal-oral contact. While 90-95% of the people may not show any symptoms, there some who experience mild symptoms like nausea, fever, decreased appetite, constipation etc. There are three types of poliomyelitis – Abortive poliomyelitis, Non-paralytic poliomyelitis and paralytic poliomyelitis.

Paget's Disease: It mostly affects older men and women. The metabolism rate of bones is altered in the person suffering from paget's disease. Bones in the human body usually break down and rebuild themselves throughout life. The breakdown process of bones during this condition becomes faster than the rate of renewal. Consequently the bones become fragile, weak and susceptible to fractures and infection.

Disk Herniation: The bones that make up our spine are being cushioned by soft disks, which are filled with a jelly-like substance. The disks play an important role of supporting the vertebrae and keeping them in place. However a hermiated disk loses its elasticity and ruptures. When the spinal disk ruptures it gets pushed

outside its normal position causing the nerves around the area to compress. This in turn causes numbness, pain and tenderness in the area adversely affecting the functioning of the nerves and the spinal cord.

Tendinitis: tendons are the tough connective tissue cord between the muscle ad bones. Which help muscle to move the bones. Tendinitis is the inflammation of the tendons that is caused by the overuse of muscle or due to an injury. It usually affects joints of the knees, hips, elbows, shoulders, heels and wrists. It causes tenderness and pain near these joints. When tendinitis affects the elbow it is given a specific name – Tennis elbow (Lateral Epicondylitis) Wilson's Disease: Copper it the human body plays an important role of keeping the bones, nerves, collage etc., in a healthy state ad this copper is absorbed from the food that we consume. When there is excess copper buildup, the liver excretes it out of the body with the help of bile (a substance produced in the liver). Wilson's disease occurs when excess amount of copper is not eliminated from the body and starts accumulating the liver, brain and other vital organs of the body.

A depolarizing neuromuscular blocking agent is a form of neuromuscular blocker that depolarizing the motor end plate, an example is succiyl choline.

The prototypical depolarizing blocking drug is succinyl choline (suxamethonium). It is the only such drug used clinically.

Ringer solution is used in in-vitro experiments on organs or tissues such as in-vitro muscle testing. Ringer solution typically contains sodium chloride, potassium chloride, calcium chloride, and sodium bicarbonate, with the last used to balance the PH other additions can include chemical fuel sources for cells, including ATP and Dextrose, as well as antibiotic and antifungal. Aerator is used for providing oxygen supply to muscle.

The *Hibiscus* leaves 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 drugs *Hibiscus* leaves extract. The activity of the standard drug is more compare to test drugs and it is above reach with the standard drug.

The skeletal muscle activity was evaluated first by the acetylcholine of different doses like 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml and 16µg/ml and with d-tubocuraine of dose about 4µg/ml. The acetylcholine were 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 by the application of acetylcholine.

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

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

5. CONCLUSION

The *Hibiscus* leaves extract was found to good skeletal muscle activity with different concentrations. When the activity was compared between the standard drug i.e, acetylcholine and test drugs Hibiscus leaves extract. The activity of the standard drug is more compare to test drugs.

The skeletal muscle activity is evaluated by using test drugs Hibiscus leaves extract of using different doses like 1 g/ml, 10 g/ml and 100 g/ml. for both the tests drugs the response have been increased. The effect of acetylcholine and Hibiscus leaves extract (HLE) were compared and the results show the more active response with the acetylcholine rather than the Hibiscus leaves extract. This study finally concluded that the effect of Hibiscus leaves extract and combination of Hibiscus leaves extract and acetylcholine were shown good skeletal muscle activity.

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