Pharmacological Evaluation of Anti-Obesity Activity of Malvaviscus Arboreusin Diet-Induced Obesity in Rats

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Abstract

Background: Obesity is a multiplex metabolism disorder associated with excess fat accumulation. It elevated the menace of cardiovascular system ailment, diabetes mellitus, and other complications. The Present study intended to examine the anti-obesity outcome of ethyl alcohol extract of Malvaviscus arboreus leaves in higher-fatfood-brought-overweight rats. **Methods:** Leaves of Malvaviscus arboreus were shade-dried, powdered by using a mortar pestle, defatted using petroleum ether laboratory reagent by Soxhlet apparatus, and then extracted the defatted extract with 95% ethanol by Soxhlet apparatus at 70 to 75 ^{o} C. Albino laboratory rats were alienated into four groups: Control, Standard (Standard drug Orlistat-10 mg/kg High fat diet), Test-1and Test-2. The treatment was given for 42 days, that is 6 weeks, by oral route with the help of oral gavage. The muscle strength and muscle grip were assessed by using Kondziela's inverted screen maze apparatus, and body weight was recorded regularly. Blood was poised by the retro-orbital perforation method for biochemical investigation for the estimation of serum lipid profile. **Results:** The test groups, particularly test-2, demonstrated significant improvements. On the 42nd day, average muscle strength in the test-2 group 4 was (206.60 seconds), close to the standard group 2 was (232.18 seconds), while the test-1 group 3 was (154.32 seconds) and the control group 1 showed only (81.57 seconds). Final body weight was also reduced: control group 1 (248.75 grams), standard group 2 (142.13 grams), test-1 group 3 (171.13 grams), and test-2 group 4 (152.88 grams).

Keywords: Obesity, Malvaviscus arboreus, Muscle strength, Muscle grip, Kondziela's inverted screen maze apparatus, Orlistat

Key Message: This research article focuses on a new understanding of the ethanolic extract of Malvaviscus arboreus leaves, which significantly reduced body weight and improved muscle strength in high-fat food-induced obese rats. The test group exhibited outcomes comparable to the standard Orlistat group, highlighting the plant's potential anti-obesity effect through enhanced metabolic function and lipid regulation over a 42-day treatment period.

1. Introduction of obesity

Obesity is a persistent, multifaceted multifactorial and morbid disease categorized by excess adiposity, which is excess body fat that harms health. It is regarded as a foremost community health concern and is graded as the 5th foremost cause of demise globally, affecting approximately 650 million people.[1,2] Obesity is the presence of extra bodily fat. It is one of the most prevalent nutritional problems in the developed world. Obesity is diagnosed using the waist or belly circumference-to-height ratio, which should be less than half of the height,[3] along with body mass index (BMI). BMI is weightiness in kgs per tallness in m^2; the normal BMI ranges from 19.8 to 26, the overweight ranges from 26.1 to 29, and the obese ranges from more than 30. Obesity is well-defined as a BMI extra than or equivalent to 30, while overweight is defined as a BMI between 26.1 and 29.

2. Introduction of plant

Malvaviscus arboreus is an erect, perennial, deciduous herb, shrub and flowering plant of the Malvaceae family. Malvaviscus arboreus, are broadlygrownup as both garden ornamentals and therapeutic plants. This plant has several culinary, medical applications, with varied ethnomedical uses and ethnopharmacological relevance including the management and treatment of multiple health disorders including, whooping cough, injuries, fever, high blood pressure, pharyngitis, bronchitis, upset stomach, hepatic difficulties, stomach ache, diarrhoea, liver gall bladder problems, cystitis, nosebleeds, renal illnesses and urinary antiseptic as a diuretic. Flowers, fruits and leaves especially their aerial parts of Malvaviscus arboreus are also suitable for the formation of lubricants, lettuce, vegetative dyes and vegetative teas. Occasionally the stem is also practiced against rubeola, hairlessness, Seborrheic dermatitis, pediculus, thrushand bedbug. In the public of Oaxaca, it is accustomed to tranquildigestivediscomfort.[4,5,6] Malvaviscusarboreus has been accustomed in outmoded medication in Central America and Haiti. Costa Ricans practice leaf liquid extraction for bladder infections, gastroenteritis,and upset stomach. Cubans use the flower liquid extractionas a mouthwash for Pharyngitis. Dominicans put on the leaf extract to pediculus, Sebopsoriasis and injuries, and flower liquid extractionis specified to breast-fed newbornswith cold. Haitians and Mexicans sip the flower liquid extractionfor respiratory disease, gastroenteritisand tonsillopharyngitis. Hondurans sip the leaf extract for Pyrexia. The peelinoculate is used to indulgenceindigestion.[7]Apart from the decorative and therapeutic standing of Malvaviscus plants [8,9,10,11,12,13] their flowers, fruits, and leaves are correspondingly supplementary to lettuce, lubricants, and botanical formulations.[14,15]

Malvaviscus arboreus Cav., usually known as Turk's turban, ladies teardrop, Turk's Cap,[16,17,18] is a member of the hibiscus family and a common component of older landscapes around central Florida,[19] Sleeping Hibiscus because its flowers never fully open and its firmly enfoldedfloral leafthat are somewhat intensifying only at the

topmost. The strong-growing plants of Sleeping Hibiscus are up to 1 m in extent, moving ovate to sketchily heart-shaped leaves and red solitary flowers, However, this recurrent, average shrub has been also extensively cultured in numerous supplementary steamy and semitropical zones of the earth. It is common worldwide in steamy regions. The plant is innate to, Central America, South America, the South-eastern United States, and Mexico[20]but has also been presented to other steamy and semitropical regions in Asia, Africa, Australia, Pacific lands and India's Uttarakhand region.

Figure: 2.1 Malvaviscus arboreus plant

3. Phytoconstituents of Malvaviscus arboreus and medicinal activity

Malvaviscus is a minor genus in the Mallow family, and several specialized metaboliteswith significant living and financial ideals have been found in this genus. Malvaviscus arboreus flowers are a substantialfoundation of phytoconstituents, including para-coumaric acid, ferulic acid, chlorogenic acid, gallic acids, protocatechuic acid, and para-hydroxybenzoic acid. Total soluble flavonoids, including kaempferol, myricetol, quercetin, and rutoside[21]are present in the flowers. Additional phytochemicals found in this plant include flavonoids: (apigenin, cyanidin, trifolin, astragalin, Sophoraflavonoloside and Leucoside),[22] quinones, phenols, phenolic acids: (gallic, protocatechuic, and para-hydroxybenzoic, beta-resorcylic, caffeic, and 4-hydroxyphenylacetic acid), cinnamic acid and/or hydroxycinnamic acids derivatives: (chlorogenic, para-coumaric ferulic and synaptic acid),[23] tannins or tannin coumarins, alkaloids, cardenolides and/or nitrogenous compounds, coumarins, saponins, anthocyanins, steroids, glycosides, terpenoids, emodins, terpenoids, fatty acids: (octadecadienoic acid, nona-decadienoic acid derivatives) sterols, triterpenes, mucilages, vernolic acid, and volatile compounds: (eugenol and 1,4-dichlorobenzene). The medicinal qualities of this plant are due to these phytochemicals.[24,25,26,27,28,29,30] Malvaviscus arboreus produces various pharmacological activities via different extracts of this plant species such as antitussive, antioxidant, antibacterial, antimicrobial, antifungal, anti-inflammatory, anticonvulsant, anticancer properties, gastrointestinal

protective, membrane stabilizer, molluscicide, insect repellent, hepatoprotective, thrombolytic, dental growth promoter.[31,32,33,35,36,37,38]

4. Plant profile

Table: 4.1 Plant profile of Malvaviscus arboreus.[34,35]

Botanical name	Malvaviscus arboreus
Kingdom	Plantae
Subkingdom	Chlorobionta
Infraorder	Phragmoplastophyta
Superdivision	Embryophytes
Division	Magnoliophytes
Subdivision	Spermatophytes
Phylum	Tracheophytes
Class	Dicotyledons
Super order	Rosanae
Order	Malvales
Family	Malvaceae
Subfamily	Malvoidea
Tribe	Hibiscea
Genus	Malvaviscus
Species	Malvaviscus arboreus

5. Materials and methods

5.1 Collection of plant material: The Malvaviscus arboreus plant was ordered online from Seed2Plant and the order number is S2P86455.

5.2 Identification and Authentication: The recognition and authentication of plants was performed in the Department of Plant Science at Mahatma Jyotiba Phule Rohilkhand University, Bareilly. The plant was authenticated by Alok Shrivastava Sir, Professor, Department of Plant Science M.J.P. Rohilkhand University, Bareilly, 243006 U.P. and the authenticated number is RU/PLSC/24/09.

5.3 Preparation of extracts: After authentication, the leaves of Malvaviscus arboreus were separated, cleaned, and shade-dried. After shade drying, the leaves were coarsely powdered by mortar and pestle and warehoused in a well-closed, air-tightflask at room temperature. The coarse powder of Malvaviscus arboreus leaves was packed in the Soxhlet apparatus and performed the defatting process first by using petroleum ether laboratory reagent 60 to 80 degrees Celsius procured from Jaipur shop at Rajendra Nagar Bareilly U.P. and the volume of this reagent is 500 ml. For defatting process required 2 bottles after the defatting process the fatty substances were to be removed.

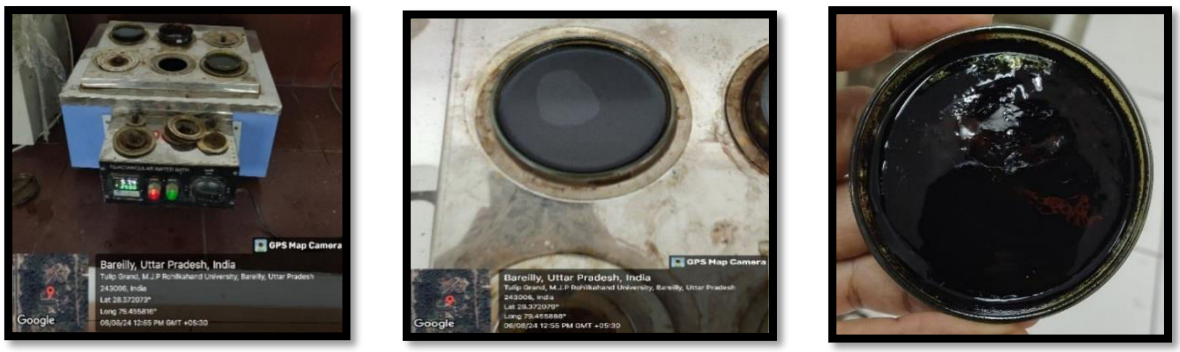
(a) (b)

Figure: 5.1 (a) Coarse powder of Malvaviscus arboreus (b) Soxhlet apparatus assembly

After defatting the plant extract, it was further continuously extracted by using 95% ethanol for 72 hours at 70 degrees celsius to obtain ethanolic extract. The ethanol is procured from the Department of Pharmacy, I.E.T. Mahatma Jyotiba Phule Rohilkhand University Bareilly U.P. For the overall extraction process used 2 liters of ethanol and 650 ml of ethanol were recovered by using blank Soxhlet apparatus assembly.

Figure: 5.2 Ethanol extract of Malvaviscus arboreus

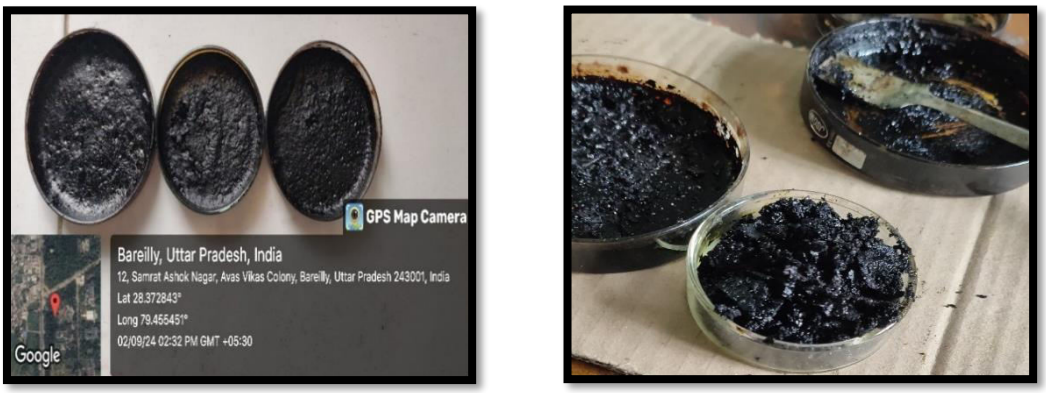
After obtaining the ethanol extract of Malvaviscus arboreus this extract was placed in the water bath at 20 to 30 degrees Celsius for making the solid consistency of this extract. When it was placed in a water bath, the ethanol extract was stirred slowly by using a glass rod that evaporates the excessive portion of ethanol in the form of vapors and the remaining plant extract was solidified at the bottom of the petri-dish and spontaneously it was converted into semi-solid consistency.



(a) **(b)** **(c)**

Figure: 5.3 (a) Ethanol Extract placed on water bath (b) Upper view of Ethanol Extract and (c) Ethanol Extract converted into semi-solid consistency

After some time, the viscous ethanol extract of Malvaviscus arboreus was placed in a hot air oven for 5 to 10 minutes at 20 degrees Celsius. The resultant ethanol extract of Malvaviscus arboreus was converted into flakes. The flakes were to be scraped by using a spatula and covered with aluminum foil to prevent microbial contamination after that the scraped solid flakes were triturated in a mortar-pestle and converted into powder form and stored in an air-tight container.



(a) **(b)**

Figure: 5.4 (a) Fully dried ethanol extract of Malvaviscus arboreus converted into flakes, and (b) Scrapped the flakes of ethanol extract of Malvaviscus arboreus

5.4. Drugs, chemicals, instruments, glasswares and other requirements: Orlistat was used as a standard drug in this study. The Active Pharmaceutical Ingredient of Orlistat was procured from Dhamtec Pharma, Maharashtra, GST Number-27AAWPM6420A1ZP. The standard dose of Orlistat was 10 mg/kg used in this study for screening the anti-obesity effect on obese rats and the duration of the study was 6 weeks, which is 42 days. In this study, only 4 rats were used in the standard group, so the amount of orlistat is required approx. 450 milligrams for 42 days, that's why the amount of orlistat A.P.I. was ordered 500 mg.

Oral gavage was used in this study for the administration of the Standard drug Orlistat, Test drug ethanol extract of Malvaviscus arboreus plant and Control vehicle 0.05 % Carboxy methylcellulose solution via oral route. The oral gavage is procured from Jiya Aviary Products, Plot No.105/106, Near Sainath Estate, Road No.18 GIDC Odhav, Ahmedabad, Gujarat, 382415, PAN No: HHIPM4337Q, GST Registration No: 24HHIPM4337Q1ZN by online mode via amazon platform. The Order Number of oral gavage was 407-1542861-3665109. The weighing machine, Soxhlet apparatus assembly, Heparinized capillary tube box, and 2 containers of petroleum ether laboratory reagent were procured from Jaipur shop at Rajendra Nagar, Bareilly U.P. For handling the experimental animals required surgical gloves. The 2 pairs of postmortem hand gloves were procured from Mehta Surgical Bareilly. Ethanol, Chloroform, Distilled water, Carboxymethylcellulose, Methanol, Glycerin, Test tubes, Test tube stand, Glass rod, Petri-dish, Filter paper, Funnel, Iron stand, water inlet and outlet pipe, Heating mantle, water bath, Round bottom flask, Beakers, Spatula, etc. was procured from the department of pharmacy, I.E.T. Mahatma Jyotiba Phule Rohilkhand University Bareilly (U.P.).

5.5 Animals: Male Albino Wistar rats,six to eight weeks old, weighing about 170 grams, were used in each group for anti-obesity activity because this strain is more susceptible to inducing obesity via a high-fat diet, and they are procured from I.V.R.I. Bareilly U.P. The I.A.E.C. permitted the protocols of study for the persistence of controlling and supervising experiments on animals. The I.A.E.C. approved 16 animals according to CPCSEA guidelines for study and provided the I.A.E.C. number for the procurement of these animals from I.V.R.I. The I.A.E.C. number is MJPRU/PY/IAEC/24/22. After the procurement of animals from I.V.R.I. first, they are acclimatized in the Animal house of the Department of Pharmacy, I.E.T., Mahatma Jyotiba Phule Rohilkhand University, Bareilly, U.P. for two weeks, which is 14 days after the acclimatization period these animals were used for experimental work. These animals were sustained in a well-ventilated room with a diurnal cycle.

5.6Protocols of experiment: The animals were alienated into 4 groups, each having 4 rats. The treatment is given in this table.

Table: 5.1 Experimental design

Groups	Treatment	Route of administration
Control Group	Normal saline	p.o.
Standard Group	Orlistat (10mg/kg) of body weight	p.o.
Test Group-1	Plant extract (100mg/kg) of body weight	p.o.
Test Group-2	Plant Extract (200mg/kg) of body weight	p.o.

5.7 Apparatus: Use the Kondziela inverted screen apparatus and the name of the test is based on the name of the apparatus, called the Kondziela inverted screen test. Kondziela invented the reversed screen assessment and issued it in 1964. It is a test of force strength using all four limbs. The Kondziela reversed screen is a 43 $cm^2$of wire netting comprising of twelve $mm^2$of 1mmthickness wire. It is enclosed by a 4 cm deeply embedded wooden border, which avoids animals from moving uphill onto the other side. The test was done by engaging the rat in the center of a wire net screen which was interchanged to an upturned site over 120 seconds, with the rat's trad falling foremost. The period when the rat dwindled the screen was renowned. This apparatus was manufactured with the help of a carpenter in Bareilly. The requirements of this apparatus are Wood, Wire mesh of Stainless steel whose diameter is 1 mm, Iron nails.

Figure: 5.5 Kondziela inverted screen maze apparatus

Procedure for the test: fetch the rats to the investigational room 5 to 20 minutes earlier testing to confirm they are properly conscious. To permit retrieval of muscularity and a return to typical levels of awakening, relaxation the rats by returning to the enclosure afterward respective test. These rats were first administered with different interventions which is standard drug orlistat, test drug ethanolic plant extract of Malvaviscus arboreus and control drug 0.05% carboxy methylcellulose solution via oral route by using oral gavage. Place the rat after the 1 hour of interventions (that is control group, standard group, and test group-1 and 2) in the center of the wire net screen, begin a stopwatch, and switch the screen to areversed site over 2 seconds, with the rat's skullfalling foremost. Embrace the screen progressively 40 to 50 cm in the airanembellished surface. Note the period of time when the rat declines, or eradicate it once the measured time of 60 seconds is touched. Extensive measured periods may be beneficial for some researches.

Scoring the reversed screen when the time of measurement is 60 seconds:

- Falling between 1 to 10 seconds gives one scoring.
- Falling between 11 to 25 seconds gives two scoring.

• Falling between 26 to 60 seconds gives three scoring.
• Falling after 60 seconds gives four scoring.

Scoring the inverted screen when the time of measurement is 120 seconds:
• Falling between 1 to 10 seconds gives one scoring.
• Falling between 11 to 25 seconds gives two scoring.
• Falling between 26 to 60 seconds gives three scoring.
• Falling between 61 to 90 seconds gives four scoring.
• Falling after 90 seconds gives five scoring.

5.9 Blood serum analysis: for the estimation of serum lipid profiles, blood was collected from overnight fasted animals, anesthetized with diethyl ether, and done retro-orbital puncture method. Blood was collected in vials and transferred to the Vet care pathology lab for the assessment of the Serum lipid profile test.

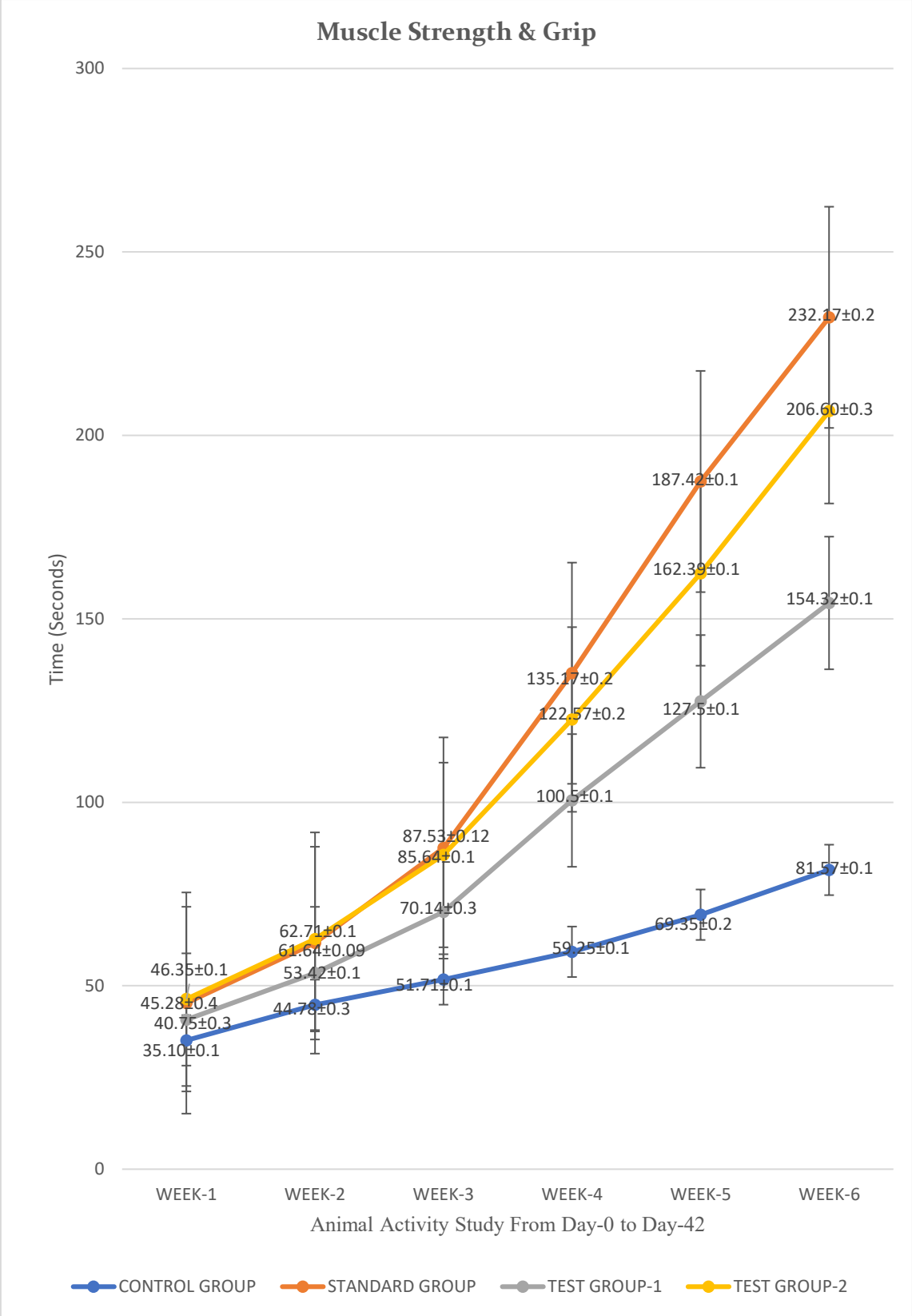
6. Result and discussion

6.1 Average for assessment of muscle strength and muscle grip by using the Kondziela Inverted Screen Maze Apparatus-Control, Standard, Test Group-1 and Test Group-2.

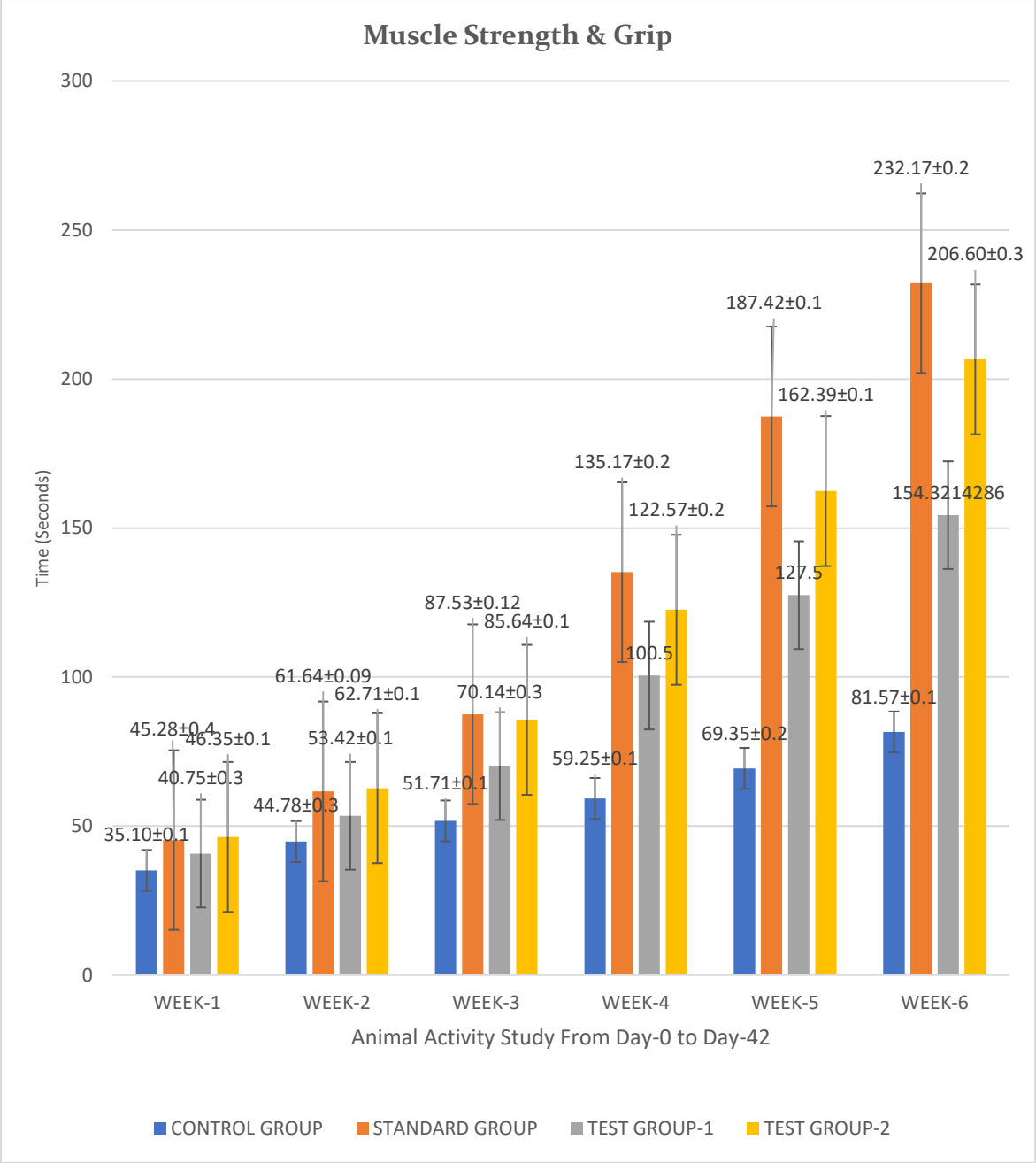
Table: 6. 1. Average readings of different groups of animals (W-Week).

Groups	W-1	W-2	W-3	W-4	W-5	W-6
Control	35.10±0.1	44.78±0.3	51.71±0.1	59.25±0.1	69.35±0.2	81.57±0.1
Standard	45.28±0.4	61.64±0.09	87.53±0.12	135.17±0.2	187.42±0.1	232.17±0.2
Test-1	40.75±0.3	53.42±0.1	70.14±0.3	100.5±0.1	127.5±0.1	154.32±0.1
Test-2	46.35±0.1	62.71±0.1	85.64±0.1	122.57±0.2	162.39±0.1	206.60±0.3

Control group animals showed inferior muscle strength and muscle grip with respect to time in seconds when compared to the standard group, test group-1 and test group-2 animals. The standard group animals exhibited superior muscle strength and muscle grip in the overall study of 6 weeks in comparison to test group-1, test group-2 and control group animals. The test group-2 animals exhibit increased muscle strength and muscle grip in comparison to test group-1 animals. All data analysed by using paired t-test, showed there is a significant difference between the control group vs standard group; standard group vs test group-1; standard group vs test group-2 animals.


Muscle Strength & Grip
300
250
200
150
100
50
0
Time (Seconds)
WEEK-1
WEEK-2
WEEK-3
WEEK-4
WEEK-5
WEEK-6
Animal Activity Study From Day-0 to Day-42
CONTROL GROUP
STANDARD GROUP
TEST GROUP-1
TEST GROUP-2
46.35±0.1
45.28±0.4
40.75±0.3
35.10±0.1
62.71±0.1
61.64±0.09
53.42±0.1
44.78±0.3
87.53±0.12
85.64±0.1
70.14±0.3
51.71±0.1
135.17±0.2
122.57±0.2
100.5±0.1
59.25±0.1
187.42±0.1
162.39±0.1
127.5±0.1
69.35±0.2
232.17±0.2
206.60±0.3
154.32±0.1
81.57±0.1

(a)



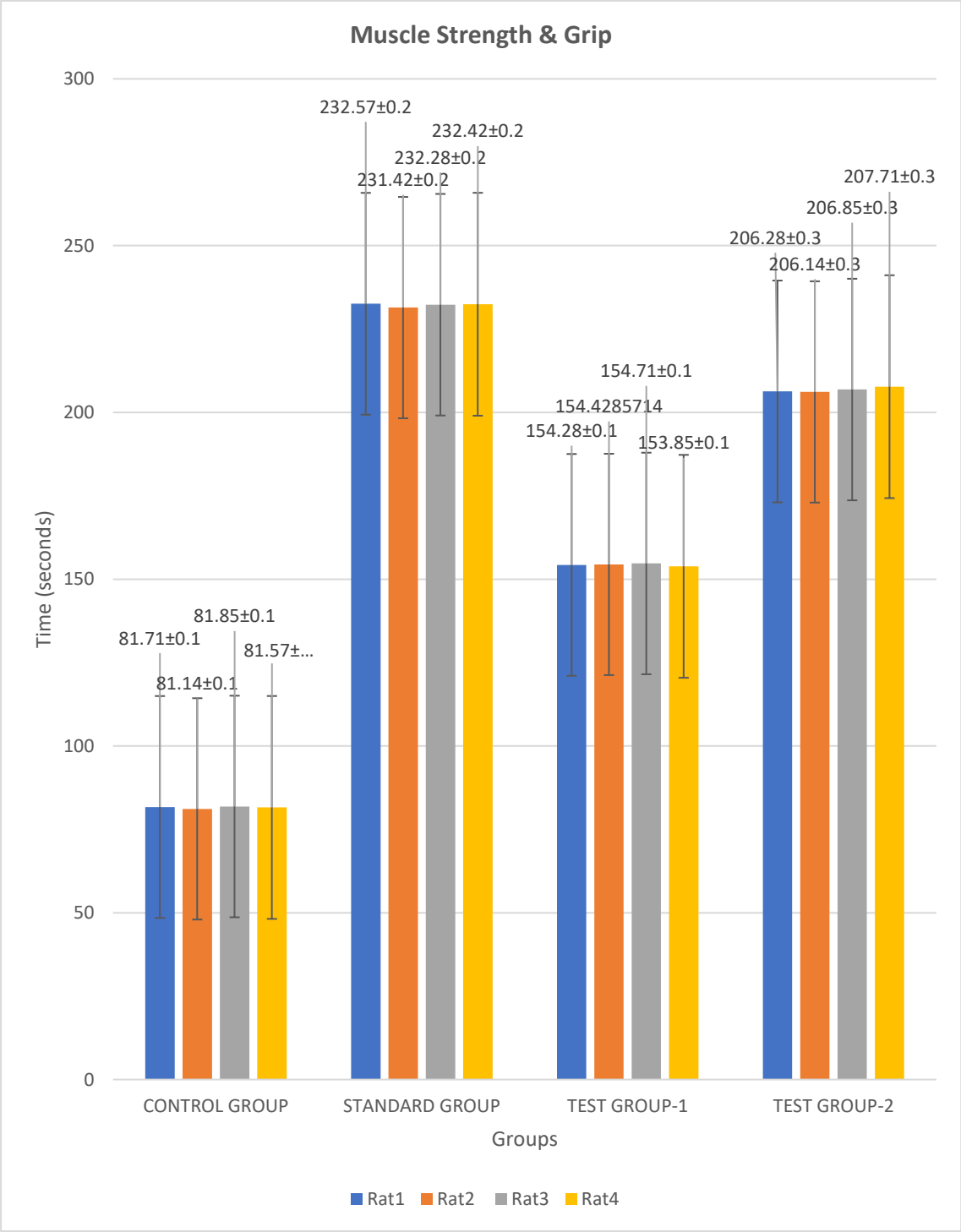
(b)

Graph: 6.1 (a) & (b) Average of Control, Standard, Test Group-1 and Test Group-2 animals for Muscle Strength and Muscle Grip from day 1 to 42-Apparatus Data. All the values were expressed as Mean ± SEM (n=4). Statistical analysis was carried out using paired t-test. The statistical significance was considered at $P < 0.05$.*: $p < 0.05$ v/s control, #: $p < 0.05$ v/s standard, @: $p < 0.05$ v/s test-1, $: $p < 0.05$ v/s test-2.

6.2 Average of6thweek for evaluation of muscle strength and muscle grip-Control, Standard, Test Group-1 and Test Group-2

Table: 6.2 Average readings of 6th week of different group of animals (S.D. Standard Deviation).

Animals	Control group	Standard group	Test group-1	Test group-2
Rat1	81.71±0.1	232.57±0.2	154.28±0.1	206.28±0.3
Rat2	81.14±0.1	231.42±0.2	154.42±0.1	206.14±0.3
Rat3	81.85±0.1	232.28±0.2	154.71±0.1	206.85±0.3
Rat4	81.57±0.1	232.42±0.2	153.85±0.1	207.71±0.3
Average	81.57	232.17	154.32	206.75
S.D.	0.30	0.51	0.35	0.71

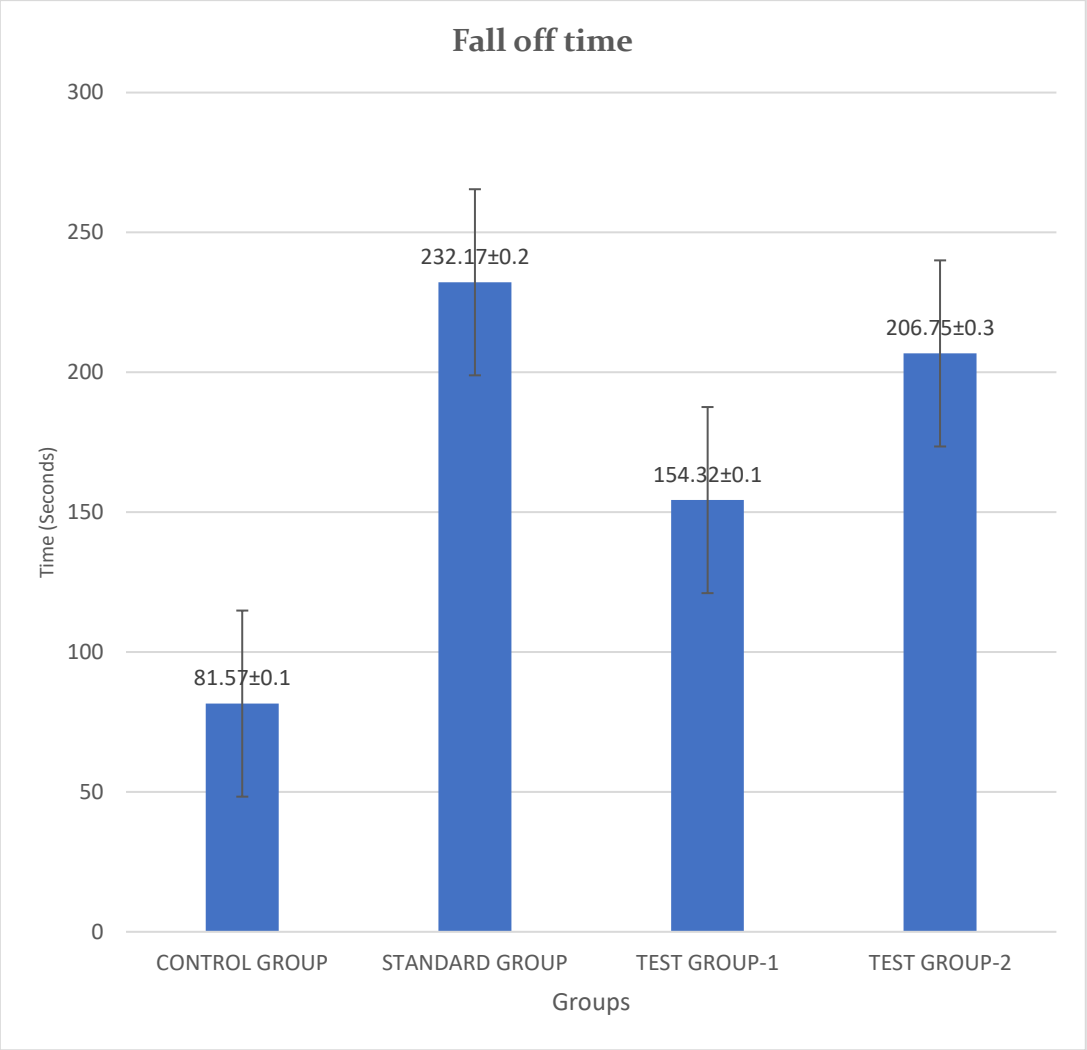


Graph: 6.2 Average of 6th week-Graph of Control, Standard, Test Group-1 and Test Group-2 animals for Muscle Strength and Muscle Grip from day 1 to 42. All the values were expressed as Mean ± SEM (n=4). Statistical analysis was carried out using paired t-test. The statistical significance was considered at P < 0.05.*: p < 0.05 v/s standard, #: p < 0.05 v/s control.

6.3 Average reading for the average of 6th week for evaluation of muscle strength and muscle grip by using Kondziela Inverted Screen Maze Apparatus-Control, Standard, Test Group-1 and Test Group-2

Table: 6.3 Average reading of 6th week of Control, Standard, Test Group-1 and Test Group-2 animals for Muscle Strength and Muscle Grip-Apparatus Data

Groups	Control group	Standard group	Test group-1	Test group-2
Fall off time	81.57±0.1	232.17±0.2	154.32±0.1	206.75±0.3



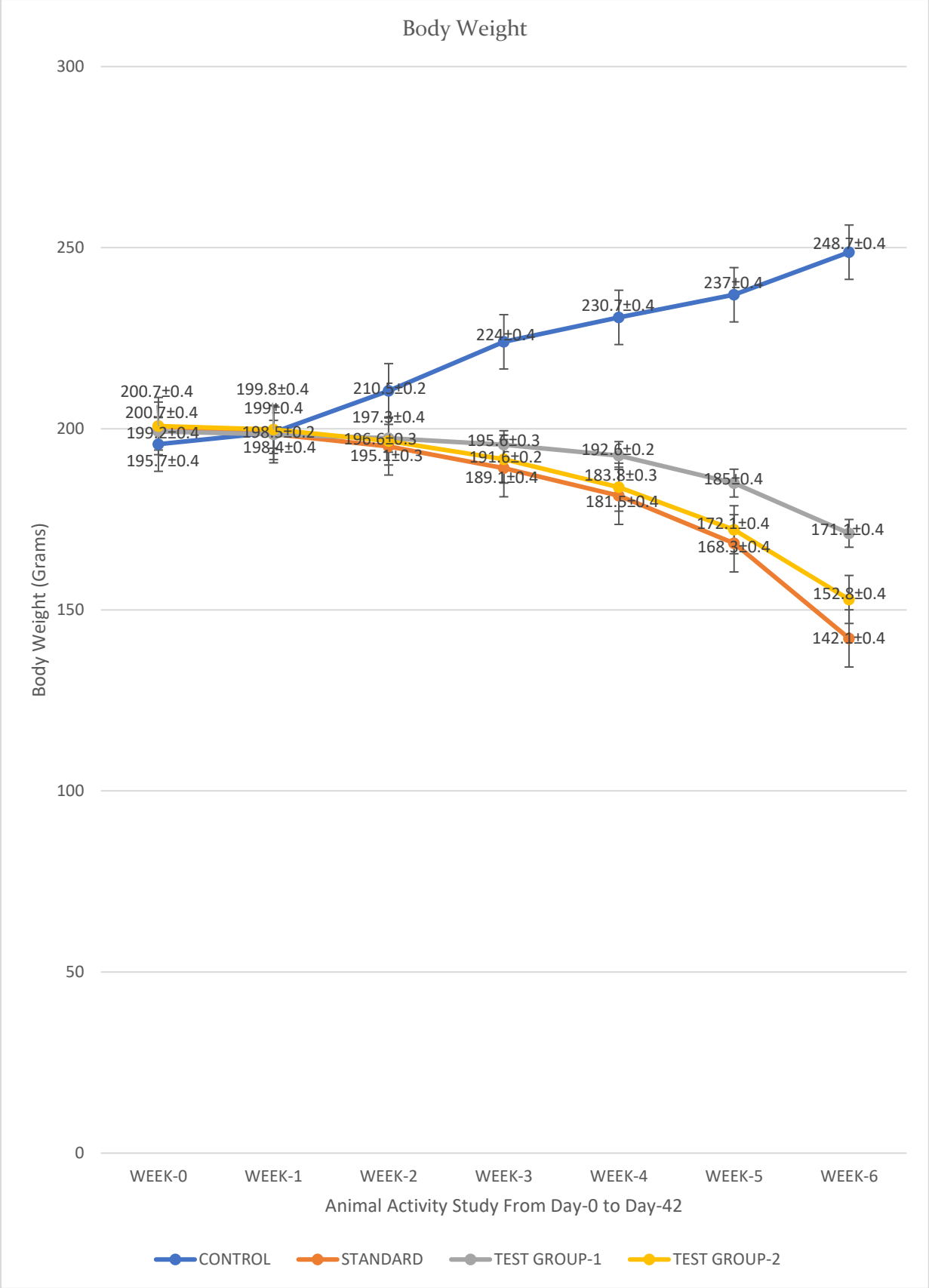
Graph: 6.3. Average reading for the average of the 6th week-Graph of Control, Standard, Test Group-1 and Test Group-2 animals for Muscle Strength and Muscle Grip Day 1 to 42-Apparatus Data**.** All the values were expressed as Mean ± SEM (n=4). Statistical analysis was carried out using paired t-test. The statistical significance was considered at P < 0.05.*: p < 0.05 v/s control, #: p < 0.05 v/s standard, @: p < 0.05 v/s test-1, $: p < 0.05 v/s test-2.

6.4 Body weight of control, standard, test group-1 and test group-2 animals

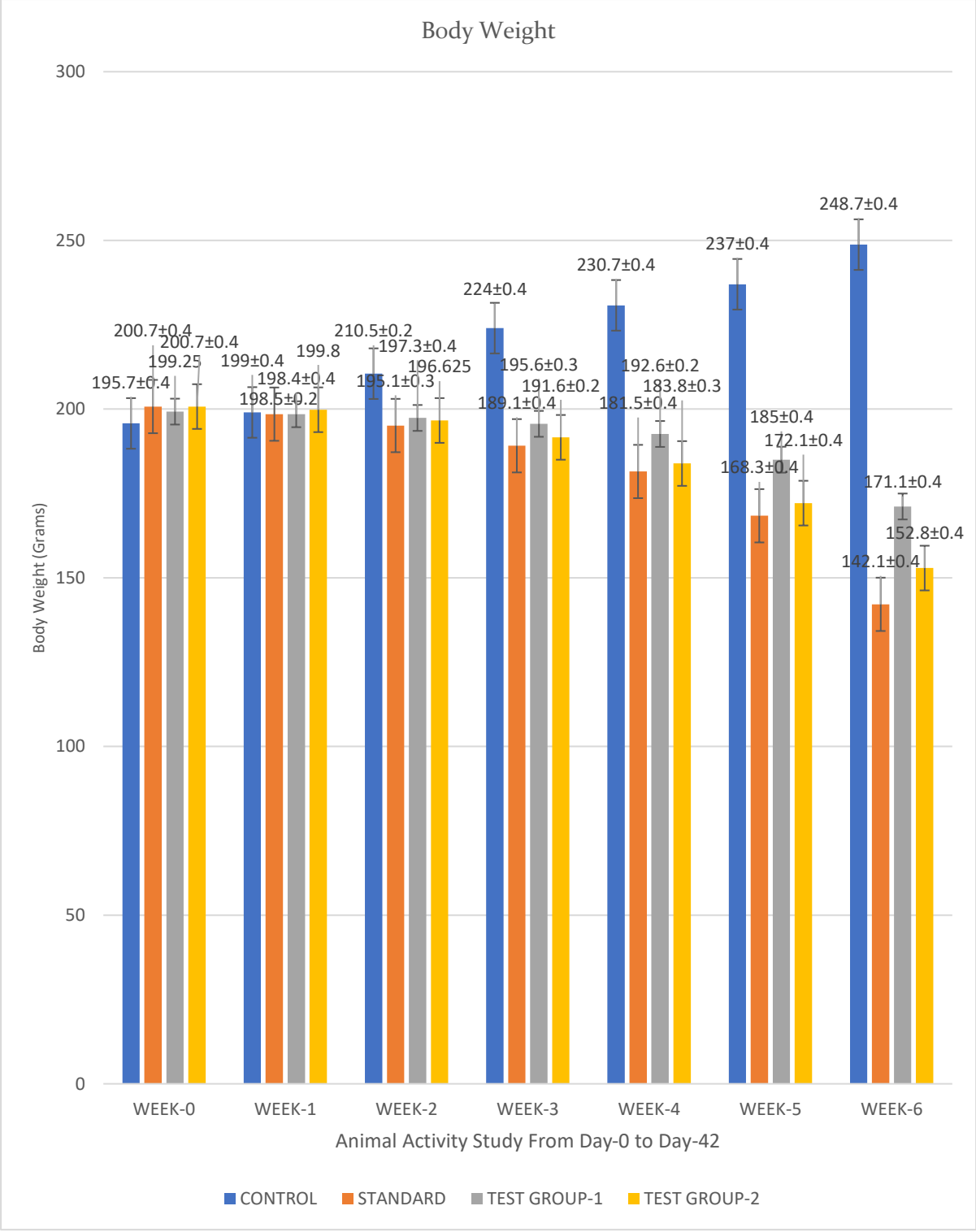
Table: 6.4 Body weight data of different groups of animals from day 0 to 42 (W-Week).

Groups	W-0	W-1	W-2	W-3	W-4	W-5	W-6
Control	195.7±0.4	199±0.4	210.5±0.2	224±0.4	230.7±0.4	237±0.4	248.7±0.4
Standard	200.7±0.4	198.5±0.2	195.1±0.3	189.1±0.4	181.5±0.4	168.3±0.4	142.1±0.4
Test-1	199.2±0.4	198.4±0.4	197.3±0.4	195.6±0.3	192.6±0.2	185±0.4	171.1±0.4
Test-2	200.7±0.4	199.8±0.4	196.6±0.3	191.6±0.2	183.8±0.3	172.1±0.4	152.8±0.4

Control group animals showed higher body weight when compared to the standard group, test group-1 and test group-2 animals. The standard group animals exhibited lower body weight in the overall study of 6 weeks in comparison to test group-1, test group-2 and control group animals. The test group-2 animals showing lowered body weight in comparison to the test group-1 animals. All data analysed by using paired t-test, showed there is a significant difference between the control group vs standard group; standard group vs test group-1; standard group vs test group-2 animals.


Body Weight
300
250
200
150
100
50
0
Body Weight (Grams)
200.7±0.4
200.7±0.4
199.2±0.4
195.7±0.4
199.8±0.4
199±0.4
198.5±0.2
198.4±0.4
210.5±0.2
197.3±0.4
196.6±0.3
195.1±0.3
224±0.4
195.6±0.3
191.6±0.2
189.1±0.4
230.7±0.4
192.6±0.2
183.8±0.3
181.5±0.4
237±0.4
185±0.4
172.1±0.4
168.3±0.4
248.7±0.4
171.1±0.4
152.8±0.4
142.0±0.4
WEEK-0
WEEK-1
WEEK-2
WEEK-3
WEEK-4
WEEK-5
WEEK-6
Animal Activity Study From Day-0 to Day-42
CONTROL
STANDARD
TEST GROUP-1
TEST GROUP-2

(a)



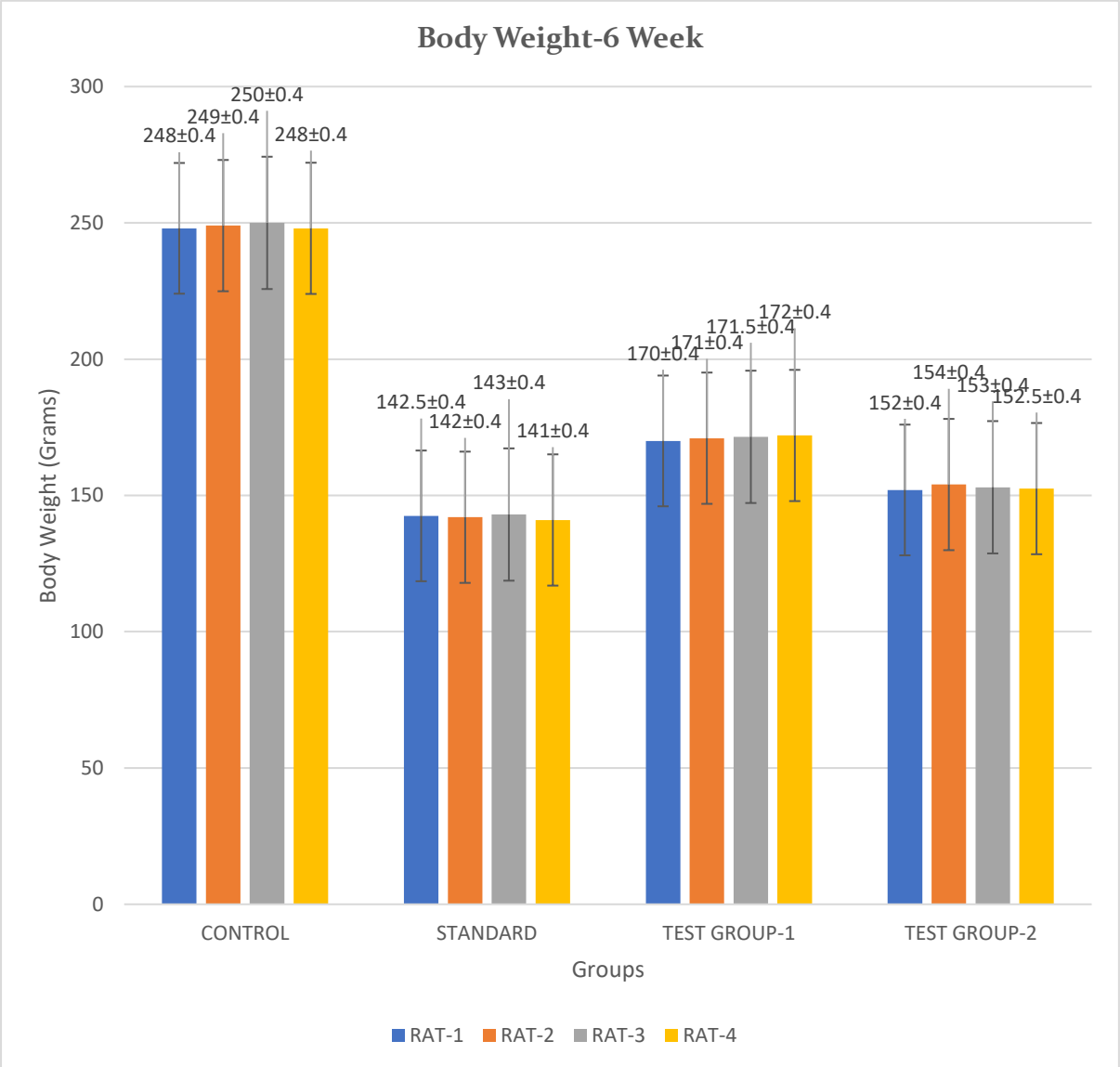
(b)

Graph: 6.4(a) and (b) Average Body Weight Data-Graph of Control, Standard, Test Group-1 and Test Group-2 animals from day 0 to 42. All the values were expressed as Mean ± SEM (n=4). Statistical analysis was carried out using paired t-test. The statistical significance was considered at $P < 0.05$.*: $p < 0.05$ v/s control, standard, test-1 and test-2 of week-1 vs. #: $p < 0.05$ v/s control, standard, test-1 and test-2 of week-0.

6.5Body weight data-6th week of control, standard, test group-1 and test group-2 animals

Table: 6.5 Body weight data of 6th week of different groups of animals (S.D. Standard Deviation).

Animals	Control	Standard	Test group-1	Test group-2
Rat-1	248±0.4	142.5±0.4	170±0.4	152±0.4
Rat-2	249±0.4	142±0.4	171±0.4	154±0.4
Rat-3	250±0.4	143±0.4	171.5±0.4	153±0.4
Rat-4	248±0.4	141±0.4	172±0.4	152.5±0.4
Average	248.75	142.12	171.12	152.87
S.D.	0.95	0.85	0.85	0.85

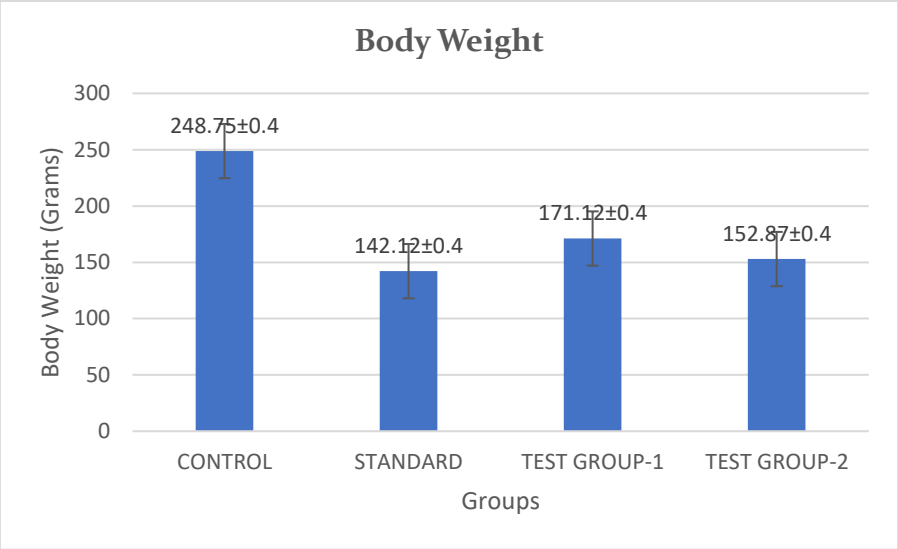


Graph: 6.5 Body Weight Data of 6th week -Graph ofdifferent group of animals from day 0 to 42.

6.6 Average reading for the average of body weight data-6th week of control, standard, test group-1 and test group-2 animals

Table: 6.6 Average reading for the average body weight of the 6th week of Control, Standard, Test Group-1 and Test Group-2 animals

Animal	Control	Standard	Test group-1	Test group-2
Average	248.75±0.4	142.12±0.4	171.12±0.4	152.87±0.4



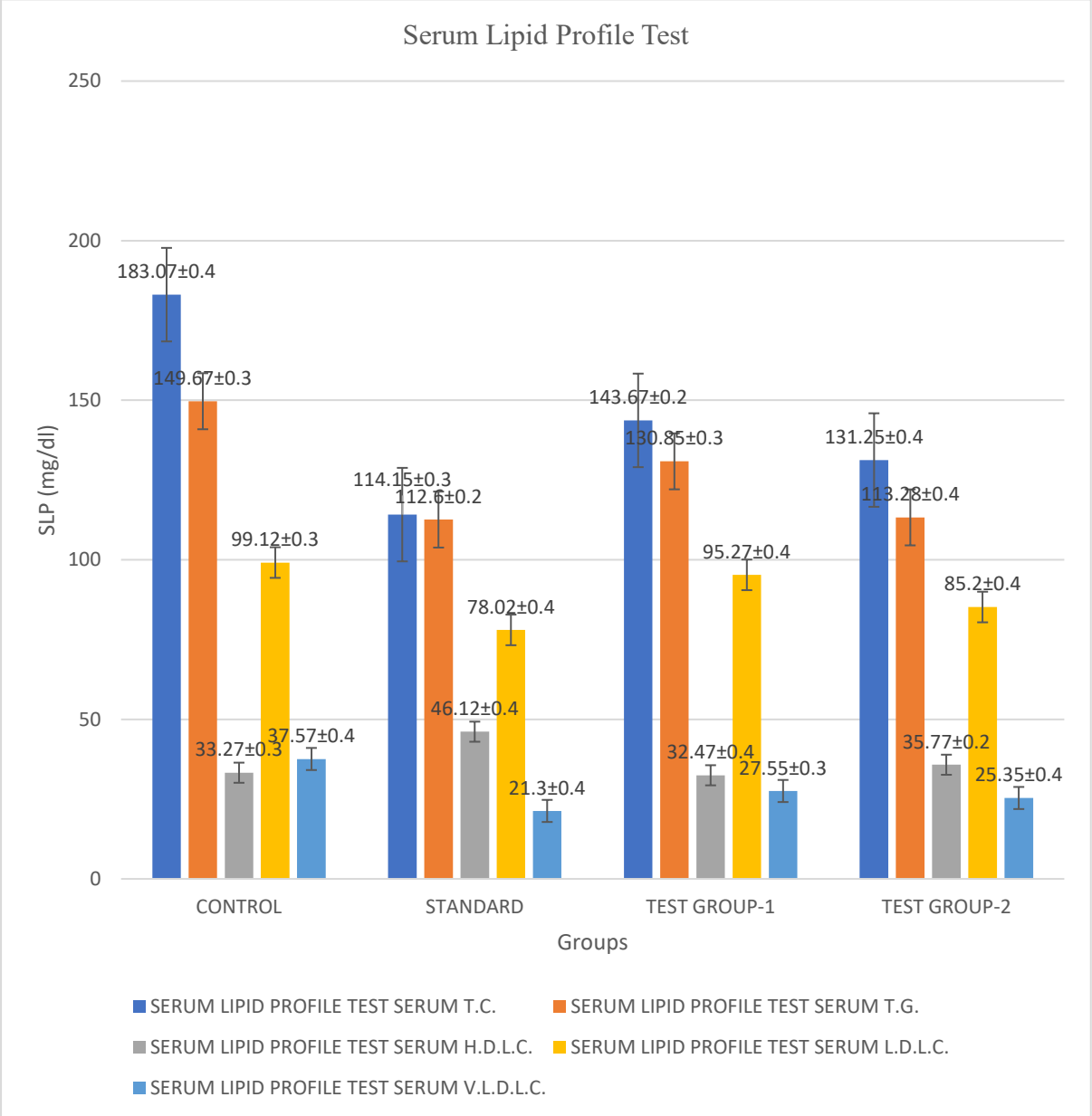
Graph: 6.6 Average reading for the average of weight data of 6th week-Graph of Control, Standard, Test Group-1 and Test Group-2 animals from day 0 to 42. All the values were expressed as Mean ± SEM (n=4). Statistical analysis was carried out using paired t-test. The statistical significance was considered at $P < 0.05$. *: $p < 0.05$ v/s standard, #: $p < 0.05$ v/s control.

6.7 Biochemical parameters data-Blood serum lipid profile analysis of control, standard test group-1 and test group-2 animals

Table: 6.7 Blood Serum lipid profile test of different group of animals.(T.C. Total Cholesterol; T.G. Triglyceride; H.D.L.C. High-Density Lipoprotein Cholesterol; L.D.L.C. Low-Density Lipoprotein Cholesterol; V.L.D.L.C. Very-Low Density Lipoprotein Cholesterol)

Groups	Average of serum lipid profile test				
	Serum T.C.	Serum T.G.	Serum H.D.L.C.	Serum L.D.L.C.	Serum V.L.D.L.C.
Control	183.07±0.4	149.67±0.3	33.27±0.3	99.12±0.3	37.57±0.4
Standard	114.15±0.3	112.6±0.2	46.12±0.4	78.02±0.4	21.3±0.4
Test-1	143.67±0.2	130.85±0.3	32.47±0.4	95.27±0.4	27.55±0.3
Test-2	131.25±0.4	113.28±0.4	35.77±0.2	85.2±0.4	25.35±0.4

Control group animals showed a higher serum lipid profile when compared to the standard group, test group-1 and test group-2 animals. The standard group animals exhibited a lowered serum lipid profile, that is Serum T.C., Serum T.G., Serum L.D.L.C. and Serum V.L.D.L.C., and increased Serum H.D.L.C., in the overall study of 6 weeks in comparison to test group-1, test group-2 and control group animals. The test group-2 animals showed a lowered serum lipid profile, that is, Serum T.C., Serum T.G., Serum L.D.L.C. and Serum V.L.D.L.C., and increased Serum H.D.L.C., in comparison to the test group-1 animals. All data analysed by using paired t-test, showed there is a significant difference between the control group vs standard group; standard group vs test group-1; standard group vs test group-2 animals.



Graph: 6.7 Serum lipid profile test of control, standard test group-1 and test group-2 animals

6.8 Discussion

The ongoing study was intended to examine the anti-obesity probable of Malvaviscus arboreus leaf extract in high-fat diet-induced obese rats. Obesity was empirically persuaded by feeding animals a fat-rich diet, which is a recognized model to mimic human obesity and metabolic alterations, including lipid profile disturbance, fat accumulation, and reduced muscular strength.

The leaves of Malvaviscus arboreus were collected, shade-dried, and pulverized to a size lessening using a mortar and pestle. The fine pulverized material was successively extracted using the Soxhlet apparatus, where defatting was first performed with petroleum ether to eradicate non-polar impurities such as lipids and waxes. Afterward, the defatted plant material experienced exhaustive extraction with 95% ethanol, which is known to extract polar and semi-polar phytochemicals, including flavonoids, polyphenols, alkaloids, saponins, and glycosides. The following ethanolic extract was concentrated and dried using a water bath and oven drying to ensure complete solvent evaporation. The dried extract was finally scraped and stored in powder form for dosing.

For the pharmacological evaluation, animals were alienated into 4 groups: Normal Control, Standard (Orlistat-a known pancreatic lipase inhibitor), and two extract-treated groups that is Test-1 group and Test-2 group, which received increased doses (100 mg/kg and 200 mg/kg) respectively of the Malvaviscus arboreus extract. Over 42 days, daily administration was conducted, accompanied by uninterrupted oversight of physical parameters, including body weight, muscle grip, and muscle strength using Kondziela's inverted screen test.

The outcome demonstrated that the extract-treated groups exhibited notably improved weight regulation and enhanced muscle strength and muscle grip compared to the calorific diet control group. The extract indicated a dose-dependent decrease in body weight gain, which indicates potential anti-obesity effects may be accredited to the phytoconstituents existing in the plant, such as flavonoids, polyphenols, and terpenoids compounds known for their lipid-dropping and antioxidant effect.

Furthermore, biochemical analyses of blood samples collected at the conclusion of the study time indicated normalization of lipid profiles, incorporating a decrease in T.C., T.G., LDL and VLDL levels in extract-treated groups related to the obese control. These consequences recommend the extract's impact on lipid metabolism, which is perhaps mediated through pancreatic lipase inhibition, reduced fat absorption, or enhanced lipid catabolism.

When compared with the standard drug Orlistat, the higher dose of Malvaviscus arboreus extract exhibited analogous efficacy, emphasizing its potential as a safe and natural alternative for administration.

7. Conclusion

The research outcome confirms that the ethanolic extract of Malvaviscus arboreus leaves exhibited anti-obesity activity in fat-rich diet-induced obese animal models. The extract inhibited extreme weight gain and also improved muscle strength, muscle grip and regulated lipid profiles, recommending a diverse effect on obesity-related parameters. The analogous results of the extract-treated groups that is Test-1 and Test-2 group and the standard Orlistat group, indicate that the plant extract could offer a plant-based, safe therapeutic alternative with reduced side effects than synthetic anti-obesity drugs. The recorded potency could be assigned to the manifestation of bioactive phytoconstituents, which may exert their effects via pancreatic lipase inhibition, fat metabolism modulation, and antioxidative mechanisms. Subsequent analysis, consisting of phytochemical profiling, mechanism-based examination, and clinical evaluations, is justified to validate Malvaviscus arboreus as a potent anti-obesity plant and to explore its probable in the regulation of overweight-associated biotransformation diseases.

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