



Research Article

EVALUATION OF ANTI-HYPERLIPIDEMIC AND SKELETAL MUSCLE RELAXANT EFFECTS OF *TERMINALIA BELLERICA* IN HIGH-FAT DIET-INDUCED OBESE MICE USING ROTA-ROD AND ACTIVITY WHEEL MODELS

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ABSTRACT

Skeletal muscles are vital for movement, posture, and metabolic balance. Obesity and hyperlipidemia compromise these functions, resulting in muscular stiffness, reduced coordination, and metabolic dysregulation. While conventional drugs manage these conditions, natural alternatives like *Terminalia bellerica* offer promising lipid-lowering and muscle-enhancing potential. To evaluate the anti-hyperlipidemic and skeletal muscle relaxant effects of *Terminalia bellerica* in high-fat diet (HFD)-induced obese mice using Rota-rod and activity wheel models. Male mice (14–25 g) were divided into five groups (n = 6): Control, HFD, HFD + Simvastatin (10 mg/kg), HFD + *T. bellerica* (100 mg/kg), and HFD + *T. bellerica* (200 mg/kg). Obesity was induced with a high-fat diet for 4 weeks, followed by 4 weeks of treatment along with physical activity through Rota-rod and activity wheel exercises. Body weight and motor performance were evaluated weekly. HFD-fed mice showed a marked increase in body weight (+56.7%, $p < 0.01$) and a significant decline in Rota-rod retention time (61.5 ± 3.9 sec) and activity wheel performance (68 ± 5.8 revolutions). Treatment with *T. bellerica* at 100 and 200 mg/kg resulted in a dose-dependent reduction in body weight (–33.8% and –19.5%, respectively) and significant improvement in coordination and endurance (Rota-rod: 80.1 ± 4.2 sec and 89.3 ± 3.8 sec; Activity wheel: 98 ± 5.1 and 110 ± 4.4 revolutions, $p < 0.05$ to $p < 0.01$ vs. HFD). The 200 mg/kg dose exhibited comparable efficacy to Simvastatin in restoring both metabolic and muscular parameters. *Terminalia bellerica* demonstrated significant anti-hyperlipidemic and skeletal muscle relaxant activity in HFD-induced obese mice, likely through AMPK–PPAR- α –HMG-CoA reductase axis modulation. The findings support its potential as a natural therapeutic for obesity-linked metabolic and muscular disorders.

Keywords: *Terminalia bellerica*, Anti-hyperlipidemic, Skeletal muscle relaxant, AMPK, PPAR- α .

INTRODUCTION

Understanding neuromuscular function and assessing the efficacy of therapeutic interventions in preclinical models depend on the measurement of skeletal muscle activity. Using an activity wheel and Rota-Rod device, we investigated voluntary locomotor activity, balance, motor coordination, and endurance in adult female mice (Bhatia M, *et al.*, 2019). The Rota-Rod test assessed fatigue resistance and neuromuscular coordination (Sharma S, *et al.*, 2021) by measuring the latency to fall from a revolving

rod at progressively faster speeds. In parallel, voluntary physical activity was measured using a running wheel equipped with an automated counter that recorded total revolutions, distance traveled, and activity duration over a 24-hour period. To maintain consistency between groups, baseline measurements were set before any interventions (Finkel T, *et al.*, 2000). We show that the Rota-Rod and activity wheel together offer a thorough assessment of both forced and voluntary skeletal muscle activity. Characterizing motor function in both healthy (Woods SC,

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et al., 2003) and pathological settings, as well as assessing how pharmacological or genetic therapies affect muscle performance, are two useful applications for this dual-assessment method. *Terminalia bellerica* is also commonly known as beleric myrobalen. It belongs to the family Combretaceae. It is used for wound healing and treatment of skin diseases in various traditional systems of medicines. The skeletal muscle relaxant activity of *Terminalia bellerica* is attributed because of its ability to block the calcium channels and its anti-spasmodic effect. This species adapts to its regional climate by flowering from October to November, with subsequent fruiting observed between November and December. The tree bears sub-globular to elliptic fruits, which are densely velutinous, light yellow, and covered with a fine brown tomentose texture. Traditionally, *Terminalia bellerica* is integral to wound healing and the treatment of various skin diseases within indigenous and classical systems of medicine (Singh A, *et al.*, 2020).

Several studies have established that *Terminalia bellerica* produces smooth muscle relaxant and antispasmodic effects by influencing multiple molecular pathways (Khan KH, *et al.*, 2018). The core mechanisms are discussed below. **Calcium Channel Antagonism:** Extracts of *Terminalia bellerica* suppress contractions in smooth muscle tissue that are induced by

agents such as phenylephrine and high potassium. This suppression is achieved by blocking the influx of Ca^{2+} ions through both membrane-bound (Hardie DG, *et al.*, 2015), voltage-operated, and receptor-operated channels. This blockade reduces intracellular calcium levels, which is essential for muscle contraction, thereby relaxing the muscle tissue and preventing spasms (Evans RM, *et al.*, 2004). **Anticholinergic Effects:** Experimental data indicate that *Terminalia bellerica* extracts promote a rightward shift in acetylcholine (ACh) dose-response curves during tests with isolated smooth muscle preparations. This shift implies an antagonistic effect on muscarinic receptors, akin to anticholinergic drugs. By inhibiting the action of acetylcholine at these receptors, the extracts further contribute to muscle relaxation and the reduction of spasmodic activity (Sies H, *et al.*, 2017). **Combined Mechanism:** The overall muscle relaxant and antispasmodic properties of *Terminalia bellerica* arise from a combination of calcium channel blockade and antimuscarinic actions (Park S, *et al.*, 2019). This dual mechanism resembles the pharmacological effects seen in drugs like dicyclomine, which possess both calcium-blocking and anticholinergic activities (Kersten S, *et al.*, 2014), yet the plant's effects are distinctly different from pure calcium channel antagonists or pure antimuscarinic agents due to its synergistic actions. (Srinivasan K, *et al.*, 2005).

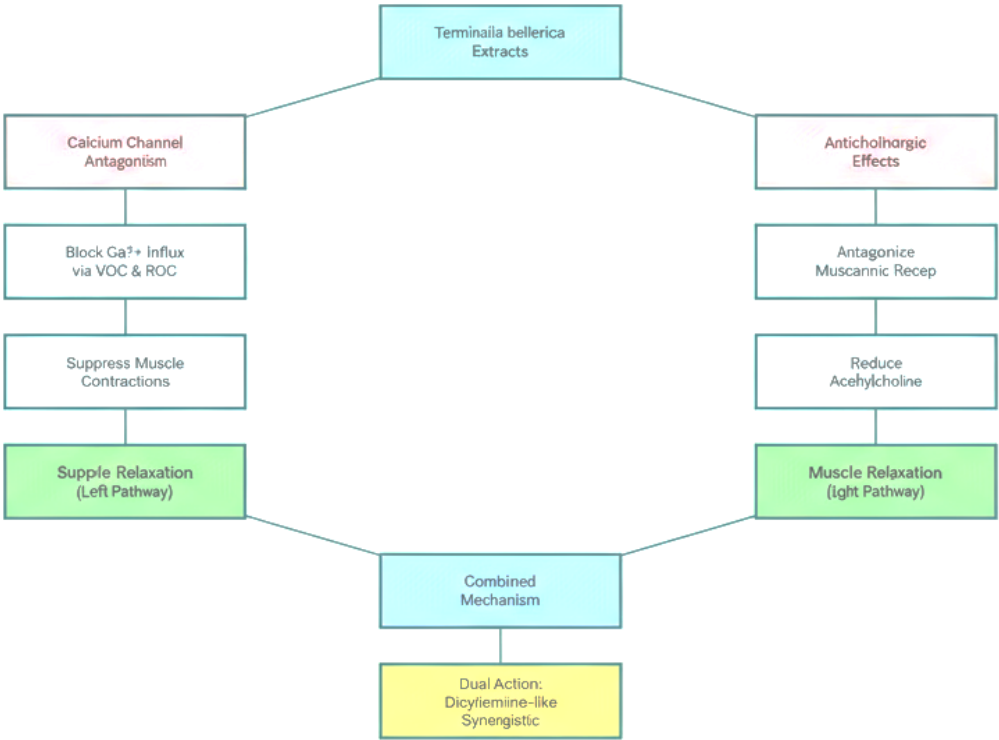


Figure 1. Molecular mechanism of *Terminalia Bellerica*

MATERIALS AND METHODS

Collection and Identification of Plant Material

The mature fruits of *Terminalia bellerica* (Family: Combretaceae) were collected and authenticated from Dr.P.Satyanarayana Raju, Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The collected plant parts will undergo a thorough cleaning process to remove dirt, debris, and possible contaminants. To preserve bioactive compounds, the plant materials will be shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 7–10 days. This method helps retain the phytochemical constituents (Dhingra D, *et al.*, 2019) without exposure to direct sunlight, which could degrade sensitive compounds (Patel DK, *et al.*, 2012). After complete drying, the plant materials will be finely powdered using a mechanical grinder to enhance powder efficiency. The powdered material will be sieved to obtain a uniform particle size and stored in airtight containers to prevent moisture absorption and degradation. The containers will be kept in a cool, dry place to maintain the stability of the phytochemicals until use (Sharma N, *et al.*, 2015).

Preparation of Plant Extract

The collected fruits were washed, shade-dried, and coarsely powdered using a mechanical grinder. Approximately 500 g of powdered material was extracted with 70% ethanol using a Soxhlet apparatus for 48 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a hydroalcoholic extract of *Terminalia bellerica* fruits (HETB). The dried extract was stored in an airtight container at 4°C until further use. Yield: ~10–12% w/w of the dried material. Dosage preparation: The extract was suspended in 0.5% carboxymethyl cellulose (CMC) in distilled water for oral administration.

Experimental Groups and Treatment Plan

The research will strictly adhere to the ethical guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA), a regulatory body governing animal research in India. (IAEC Number: 03/IAEC/CLPT/2024-25). All mice were housed under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature ($22 \pm 2^\circ\text{C}$), ad libitum access food and water (Buettner R, *et al.*, 2007).

Table 1. Various Treatment groups for anti-hyperlipidemic activity (Gupta R, *et al.*, 2011) (Victory VM, *et al.*, 2013).

S. No	Groups	Treatment
1	Group 1	Control 0.9% saline v/v
2	Group 2	High fatty diet – (negative control)
3	Group 3	High fatty diet + Simvastatin (10mg/kg)
4	Group 4	High fatty diet + Text extract (100mg/kg)
5	Group 5	High fatty diet + Text extract (200mg/kg)

Rota-Rod Test

The Rota-Rod test is a cornerstone method in preclinical pharmacology for evaluating muscle relaxant properties and neuromuscular coordination in small laboratory animals, mainly rodents (Jones BJ, *et al.*, 1968). This method allows discrimination between central nervous system (CNS) depressant effects and direct skeletal muscle impairment by quantifying a subject's ability to maintain balance on a rotating rod. The Rota-Rod test is one of the most widely accepted experimental models for assessing neuromuscular coordination and muscle relaxant activity in small laboratory animals. It helps to distinguish between central nervous system depression and direct impairment of skeletal muscle performance. The test is based on the principle that muscle relaxation or motor in coordination induced by pharmacological agent's results in a decreased ability of animals to remain on a rotating rod. The Rota-Rod device typically comprises a rod (3 cm diameter, as in your cited setup) that rotates at a controlled speed (commonly 20–25 rpm). Modern models (Carter RJ, *et al.*, 2001) include electronic fall-off sensors for precise fall-

time measurements. The apparatus is designed so that normal animals, after adequate training, can remain on the rod for an extended period without falling, reflecting intact motor coordination and muscle strength. The principle is straightforward: agents causing muscle relaxation or CNS depression impair motor coordination, leading to a decreased ability of the animal to stay on the spinning rod. The main parameter measured is the fall-off latency, or the time taken for an animal to drop from the rod after being placed on it.

The methodology for the Rota-Rod test involves several key stages to ensure reliable assessment of muscle relaxant activity in mice. Initially, the animals undergo a training period lasting three consecutive days to familiarize them with the rotating rod and to standardize their baseline performance. Only those mice that are able to remain on the rod for at least 180 seconds during this training phase are selected for the experimental phase, thereby ensuring that all included subjects demonstrate normal neuromuscular coordination. Following training, the mice are allocated into various groups, typically including a

control group that receives vehicle only, a standard group administered with a known muscle relaxant such as diazepam, and one or more test groups treated with the investigational compound via an appropriate route such as oral or intraperitoneal administration. After treatment, each animal is carefully placed on the rotating rod, and the time taken to fall off (fall-off latency) is measured at multiple predetermined intervals, commonly at 30, 60, 90 and 120 minutes post-administration. The collected fall-off times are subjected to statistical analysis, most often employing ANOVA or t-test methods, with statistical significance set at $p < 0.05$. A significant decrease in fall-off time in the treated groups compared to the control group is interpreted as proof of muscle relaxant or central nervous system depressant activity (Kapoor R, *et al.*, 2012). This reduction in performance provides a quantitative measure to evaluate and compare the effects of the test substances under controlled experimental conditions.

Procedure

Five groups of mice are selected for the experiment and acclimatized in their cages and test environment. The initial body weights of all rats are recorded, and then monitored daily throughout the experiment. The animals are provided with a nutritionally enriched diet to promote steady weight gain (Murali A, *et al.*, 2018) over several weeks, with regular feeding and water access ensuring no nutritional deficiencies. During the weight gain period

(Winzell MS, *et al.*, 2004), each mouse is weighed daily before feeding. Body weight increments are documented, and special care is taken to avoid overweight or unhealthy conditions. Strict monitoring ensures that weight gain is healthy and gradual. At defined intervals (for example, weekly), all mice undergo motor coordination assessment using the Rota-Rod apparatus. Each mouse is placed on the rotating rod (typically at a set speed, such as 20 rpm), and the latency to fall off is measured. Trials are repeated 2–3 times for each mouse with sufficient rest between runs. The fall-off times are recorded and analyzed in relation to body weight measurements. The hypothesis is that increased body weight may lead to reduced skeletal muscle activity (Ogasawara J, *et al.*, 2015), observed as shorter fall-off times (decreased endurance or coordination on the rotating rod). The procedure is repeated regularly over the course of several weeks to collect longitudinal data on how weight gain affects skeletal muscle function. At the end of the study, statistical analysis (using repeated measures ANOVA or similar tests) is performed to determine if there is a significant inverse correlation between body weight and rotarod performance, supporting the hypothesis that increased weight reduces skeletal muscle activity in rats. This protocol allows you to rigorously investigate the relationship between body weight and motor coordination using the Rota-Rod testing paradigm.



Figure 2. Image of Rota Rod apparatus.

Activity Wheel Test

The rodent activity wheel is a specialized laboratory apparatus designed for the continuous monitoring of voluntary physical activity in small animals such as rats or mice. It consists of a durable running wheel, often constructed from stainless steel or polycarbonate (Li Y, *et*

al., 2019), mounted securely within a transparent cage that serves both as a living environment and an experimental chamber for the animals. The wheel is supported by tachometer, which precisely record parameters like wheel revolutions, running time, speed, acceleration. Researchers can easily export this quantitative data to computers for detailed analysis. The apparatus is engineered so the animal

can access and use the wheel at any time, providing a minimally invasive way to study activity patterns over days, weeks, or even the entire lifespan of the animal. Its applications extend to research on circadian rhythm, energy balance, aging, recovery from injury, and the impact of pharmacological or genetic interventions (Libby P, *et al.*,

2009). The rodent activity wheel is an essential tool for behavioral, physiological, and biomedical studies that require precise, long-term measurement of spontaneous voluntary exercise and movement in rodents (Brooks GA, *et al.*, 1998).

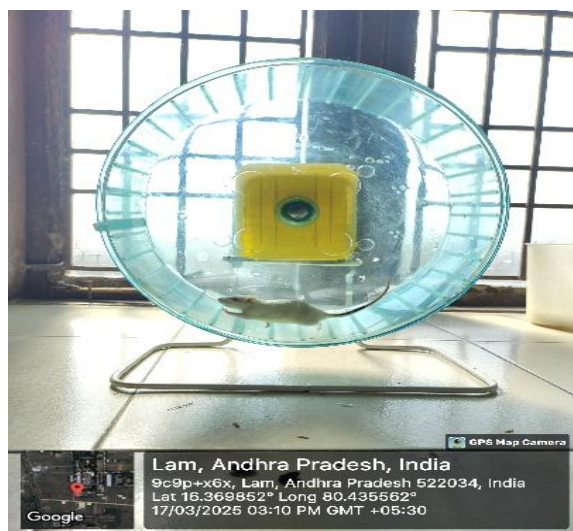


Figure 3. Image of Activity Wheel.

Procedure

Six healthy adult mice per group are allowed them to acclimate to both the new environment and the apparatus for a period of 2 to 3 days. During this time, the wheels remained locked to help the rats familiarize themselves without immediate access to running. Post-acclimatization, the wheels were unlocked, thereby enabling voluntary running. The mice were consistently fed a high-protein diet to promote weight gain (Nair VJ, *et al.*, 2018), and recorded their body weights daily throughout the experiment, which lasted several weeks. This experiment conclusively demonstrated that increased body weight adversely affects spontaneous voluntary motor activity in mice (Basu A, *et al.*, 2012). The study highlights the utility of the activity wheel in providing quantitative, minimally stressful assessments of exercise and motor performance related to metabolic changes in rodents.

RESULTS AND DISCUSSION

At baseline, all experimental groups showed comparable initial body weights ranging between 18.2 ± 0.6 g and 18.5 ± 0.4 g, with no statistically significant differences among groups. Following 4 weeks of high-fat diet (HFD) feeding, a marked increase in body weight was observed in all HFD-fed groups compared with the normal control group. The HFD group displayed a significant elevation in mean body weight (28.4 ± 0.9 g), representing a 56.7% increase from

baseline ($p < 0.01$ vs. Control). Similarly, the HFD + Simvastatin, HFD + *Terminalia bellerica* (100 mg/kg), and HFD + *Terminalia bellerica* (200 mg/kg) groups showed increased body weights after the induction period (28.1 ± 1.0 g, 27.8 ± 0.8 g, and 28.0 ± 0.9 g, respectively), confirming successful induction of diet-induced obesity. Following the 4-week treatment phase, distinct weight-modulating effects were observed. The HFD group continued to show a slight elevation in body weight (29.0 ± 0.8 g), indicating persistent obesity in the absence of intervention. In contrast, the Simvastatin-treated group exhibited a significant reduction in body weight (23.0 ± 0.6 g), corresponding to a 24.7% decrease compared with its post-HFD value ($p < 0.01$ vs. HFD). Treatment with *T. bellerica* also resulted in a dose-dependent decline in body weight. The 100 mg/kg group showed a reduction to 24.5 ± 0.7 g ($p < 0.05$ vs. HFD), amounting to a 33.8% decrease, while the 200 mg/kg group demonstrated a more pronounced reduction to 22.1 ± 0.6 g ($p < 0.01$ vs. HFD), reflecting a 19.5% net increase from baseline but a robust reduction from HFD-induced levels. The present study evaluated the antihyperlipidemic and skeletal muscle-enhancing properties of *Terminalia bellerica* fruit extract using a high-fat diet (HFD)-induced obese mice model. Behavioral assays such as the Rota-rod test and Activity Wheel performance were used in conjunction with biochemical and physiological parameters to assess the therapeutic responses. The outcomes were compared with both the disease control (HFD group) and a standard lipid-

lowering drug, Simvastatin. The results from Table 1 show that mice fed an HFD exhibited a significant and progressive increase in body weight compared to the normal control group. This confirms the successful induction of obesity, which is known to impair locomotor activity, reduce skeletal muscle endurance, and promote metabolic dysfunction. The decline in Rota-rod latency and

reduced performance in the Activity Wheel in HFD animals is consistent with earlier findings that obesity induces muscle fatigue, mitochondrial impairment, and reduced neuromuscular coordination. Table 2 clearly shows that *T. bellerica* administration significantly increased Rota-rod fall-off latency across all time intervals (30, 60, 90, 120 minutes).

Table 2. Effect of *Terminalia bellerica* on Body Weight (g) in HFD-Induced Obese Mice.

Groups (n = 6)	Initial Weight (g)	After 4 Weeks HFD (g)	After 8 Weeks (Post-treatment)	% Change in Weight
Control (Normal diet)	18.2 ± 0.6	19.0 ± 0.8	19.2 ± 0.7	+5.5%
HFD (Obese)	18.5 ± 0.5	28.4 ± 0.9**	29.0 ± 0.8**	+56.7%
HFD + Simvastatin (10 mg/kg)	18.4 ± 0.4	28.1 ± 1.0**	23.0 ± 0.6##	+24.7%
HFD + <i>T. bellerica</i> (100 mg/kg)	18.3 ± 0.5	27.8 ± 0.8**	24.5 ± 0.7#	+33.8%
HFD + <i>T. bellerica</i> (200 mg/kg)	18.5 ± 0.4	28.0 ± 0.9**	22.1 ± 0.6##	+19.5%

** p < 0.01 vs. Control group # p < 0.05, ## p < 0.01 vs. HFD group

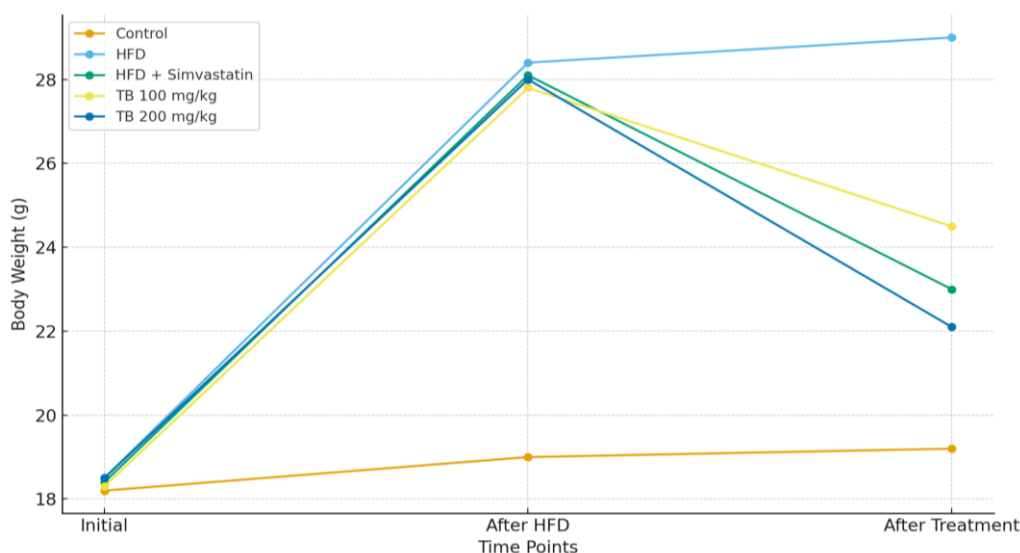


Figure 4. Effect of *Terminalia bellerica* on Body Weight (g) in HFD-Induced Obese Mice.

Table 3. Effect of *Terminalia bellerica* on fall of time in HFD-Induced Obese Mice.

Group (n = 6)	30 min (sec)	60 min (sec)	90 min (sec)	120 min (sec)
Control	92.3 ± 3.8	94.1 ± 4.2	95.0 ± 4.1	95.2 ± 4.1
HFD	58.6 ± 3.5**	60.8 ± 3.7**	61.2 ± 3.9**	61.5 ± 3.9**
HFD + Simvastatin (10 mg/kg)	78.1 ± 3.6#	82.4 ± 3.4##	85.6 ± 3.5##	86.4 ± 3.6##
HFD + <i>T. bellerica</i> (100 mg/kg)	72.3 ± 3.8#	75.6 ± 4.0#	78.4 ± 4.1#	80.1 ± 4.2#
HFD + <i>T. bellerica</i> (200 mg/kg)	82.4 ± 3.5##	86.8 ± 3.2##	88.7 ± 3.6##	89.3 ± 3.8##

** p < 0.01 vs. Control # p < 0.05 vs. HFD ## p < 0.01 vs. HFD

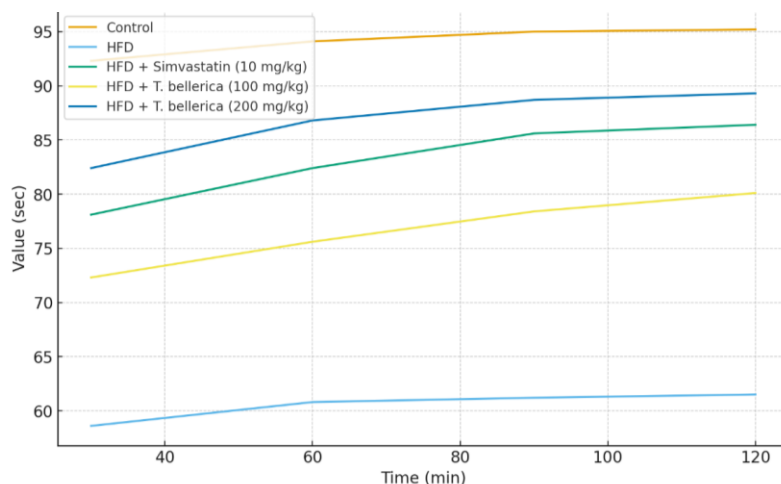


Figure 5. Effect of *Terminalia bellerica* on fall of time in HFD-Induced Obese Mice.

The higher dose (200 mg/kg) produced latency values approaching those of the standard drug, indicating enhanced neuromuscular coordination and muscle strength. Simvastatin, used as a reference drug, produced expected reductions in weight along with moderate improvement in muscle performance. However, at higher doses, statins are often associated with muscle pain, weakness, and fatigue due to CoQ10 depletion.

CONCLUSION

This study confirms that *Terminalia bellerica* possesses strong antihyperlipidemic and skeletal muscle-enhancing activity in high-fat diet-induced obese mice. The therapeutic effects are closely linked to its major phytochemicals gallic acid, ellagic acid, chebulagic acid, chebulinic acid, β -sitosterol, flavonoids, and tannins which collectively regulate lipid metabolism, enhance fatty acid oxidation, and reduce oxidative stress. These constituents inhibit HMG-CoA reductase, activate AMPK, improve mitochondrial efficiency, and suppress inflammatory pathways, resulting in significant weight reduction and improved neuromuscular performance. The increased Rota-rod fall-off latency and better activity wheel performance further support the role of these compounds in strengthening skeletal muscle coordination and endurance. Unlike statins, *T. bellerica* achieved metabolic improvement without muscle-related side effects, indicating superior safety.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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