RESEARCH PAPER

In Vitro Assessment of Telmisartan and Sorafenib in Salt-Induced Cardiac Hypertrophy in H9C2 (2-1) Cells

Anil Kumar Prajapati[1,2], Gaurang Shah[2]*

[1]Research Scholar, Gujarat Technological University, Ahmedabad, Gujarat, India
[2]Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad, Gujarat, India 380009

Corresponding author: Dr. Gaurang Shah, gauarng.shah@lmcp.ac.in

Received: 1st Apr, 2026; Revised: 22nd Jun, 2026; Accepted: 26th Jun, 2026; Available Online: 2nd July, 2026

ABSTRACT

Cardiac hypertrophy is a key pathological feature contributing to adverse cardiac remodeling and heart failure. Excessive salt intake is known to promote hypertrophic responses through activation of growth factor–mediated signaling pathways. The present study investigated and compared the effects of Telmisartan and Sorafenib on salt-induced cardiac hypertrophy using an in vitro H9C2 (2-1) cell model. High-salt exposure (240 mM NaCl) significantly increased cell surface area and α-smooth muscle actin (α-SMA) levels, confirming hypertrophy. Cell viability analysis identified non-cytotoxic concentrations of both drugs for further evaluation. Telmisartan treatment significantly and dose-dependently reduced salt-induced cellular hypertrophy at concentrations of 0.25, 0.20, and 0.15 µM, while Sorafenib significantly reduced cell size at 0.25 µM. However, neither Telmisartan nor Sorafenib produced a statistically significant reduction in α-SMA levels under the experimental conditions. These findings demonstrate that both agents effectively attenuate salt-induced cardiac hypertrophy in vitro, with Telmisartan showing a pronounced dose-dependent effect. However, neither agent significantly reduced fibrotic marker level, suggesting limited anti-fibrotic efficacy in this in vitro model.

Keywords: In vitro study, Telmisartan, Sorafenib, Cardiac hypertrophy

How to cite this article: Prajapati AK, Shah G. In Vitro Assessment of Telmisartan and Sorafenib in Salt-Induced Cardiac Hypertrophy in H9C2 (2-1) Cells. Int J Drug Deliv Technol. 2026;16(66s):745-749. DOI: 10.25258/ijddt.16.66s.70

Source of support: Nil

Conflict of interest: None

ACKNOWLEDGEMENTS

The authors are grateful to Vaibhav Analytics, Shahibaug, Ahmedabad, Gujarat, for kindly providing the gift sample of Telmisartan USP, and to CIPLA Ltd. for providing the gift sample of Sorafenib tosylate used in this study.

INTRODUCTION

Cardiac fibrosis is a key histopathological hallmark of pathological hypertrophy, in which excessive accumulation of extracellular matrix proteins promotes ventricular dilation and impairs contractile function (Kong et al. 2014). Cardiac hypertrophy is a key compensatory response to chronic increases in hemodynamic load. However, prolonged myocardial overload ultimately progresses to heart failure, a condition marked by ventricular chamber dilation, impaired contractile function, and reduced survival (Dostal 2000). In contrast, pathological hypertrophy arises from chronic pathophysiological stressors such as neurohormonal activation, aortic stenosis, inflammation, or myocardial injury. Although initially adaptive, helping to normalize wall stress and maintain contractile function, pathological hypertrophy often progresses to decompensation and heart failure (Hilfiker-Kleiner et al. 2006). Dr. Owens and his research team have highlighted the role of Angiotensin II (Ang II) as a growth factor involved in cellular hypertrophy, particularly in the cardiovascular system. The angiotensin II receptor type 1 (AT-1) has been identified as a key mediator in hypertension and cardiac fibrosis (Vukelic and Griendling 2014).

Telmisartan is an angiotensin II (Ang II) receptor antagonist widely prescribed for the clinical management of hypertension. Elevated blood pressure increases cardiac afterload and contributes to the development of cardiac hypertrophy. However, activation of ventricular Ang II receptors can occur independently of systemic hypertension. Consequently, Telmisartan may attenuate cardiac hypertrophy not only by alleviating hypertensive stress but also by directly inhibiting Ang II receptors in cardiac tissue (Li et al. 2017).

Sorafenib, a multi-kinase inhibitor, is primarily used as an antineoplastic agent. Sorafenib targets Raf serine/threonine kinase, VEGFR, PDGFR tyrosine kinases, and c-kit tyrosine kinase (Raut et al. 2022).

The high-salt model is a well-established and widely used experimental system for investigating salt-induced cardiac hypertrophy, providing valuable insights into the cardiovascular consequences of excessive salt intake (Pap et al. 2023). Moreover, elevated dietary salt consumption has been associated with increased tissue inflammation and the progression of cardiac fibrosis, further contributing to adverse cardiac remodeling (Wenzel et al. 2019).

In this study, we performed an in vitro investigation to compare the effects of Telmisartan and Sorafenib on salt-induced cardiac hypertrophy and fibrosis using H9C2 (2-1) cells. The objective of this study was to evaluate whether the growth factor inhibitor Sorafenib can attenuate salt-induced cardiac hypertrophy and fibrosis in H9C2 (2-1) cells, and to compare its effects with those of Telmisartan.

MATERIALS AND METHODS

The H9C2 (2-1) cell line was purchased from the NCCS, Pune, India. Dulbecco's Modified Eagle's Medium (DMEM; HyClone),10% fetal bovine serum (FBS; Himedia), 100 U/mL penicillin (Himedia), and 100 µg/mL streptomycin (Himedia), PBS (Himedia), Dimethyl sulfoxide (DMSO, SRL), MTT reagent (Himedia), Telmisartan USP (gift sample from Vaibhav Analytics, Shahibaug, Ahmedabad, Gujarat), and Sorafenib

tosylate (gift sample from CIPLA Ltd.). Telmisartan and Sorafenib were dissolved in 0.1% DMSO to ensure adequate solubilization. Drug treatments were applied concurrently with salt exposure for 48 hours. Fresh working solutions of both drugs were prepared immediately before use to maintain solubility and stability.

CELL VIABILITY

Cell viability was evaluated using the MTT assay. H9C2 (2-1) cells at passage number 20 were plated at a density of 2×10^3 cells per well in 96-well plates and allowed to adhere and stabilize for 24 hours until approximately 80% confluency was reached. The cells were then exposed to varying concentrations of Telmisartan and Sorafenib (0.25 µM, 0.5 µM, and 1 µM; 0.25 µM, 0.5 µM, and 1 µM) for an additional 24 hours. To induce formazan crystal formation, MTT solution (5 mg/mL) was added to each well and incubated at 37°C for 2 hours. The medium was gently removed, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Absorbance was measured at 540 nm using a Varioskan Flash ELISA plate reader (Thermo Scientific). Cell viability was calculated and expressed as a percentage relative to the untreated control group. All experiments were performed in triplicate (n = 3).

SALT-INDUCED CARDIAC CELL HYPERTROPHY AND FIBROSIS

H9C2 (2-1) cells at passage number 22 were seeded at a density of 2×10^4 cells per well in 24-well plates and incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours, allowing cell adherence and normalization of morphology until approximately 80% confluency was reached. Cardiac hypertrophy was induced in the disease control (DC) group by treating cells with a high-salt medium consisting of DMEM supplemented with an additional 130 mM NaCl, resulting in a final sodium concentration of 240 mM (Zhang et al. 2019). The normal control (NC) group received DMEM without added salt. All groups, including controls and individual drug treatments (lower concentration compared to non-cytotoxic concentration shown in Table 1), were maintained under standard culture conditions for 48 hours. Following treatment, hypertrophic changes were assessed by measuring cell surface area using an inverted microscope (Olympus, Tokyo, Japan) equipped with a QImaging Micropublisher 5.0 RTV camera and operated using QCapture Pro software (version 7). Cell surface area measurements were obtained from three independent wells per group (n = 3) and averaged for analysis. In addition, culture supernatants were collected to evaluate α-smooth muscle actin (α-SMA) levels, a marker associated with cardiac fibrosis (Holm Nielsen et al. 2019). Quantification of α-SMA was performed using a commercial ELISA kit (Krishgen Biosystem, India; Catalog No. KLR3435) according to the manufacturer's protocol (n = 3).

Table 1. Different groups of telmisartan and sorafenib based on concentration

Concertation	Telmisartan Group	Sorafenib Group
0.25 µM	T 1	S 1
0.20 µM	T 2	S 2
0.15 µM	T 3	S 3
0.10 µM	T 4	S 4
0.05 µM	T 5	S 5

STATISTICAL ANALYSIS

Statistical analysis was conducted using GraphPad Prism version 8.3.0. Group comparisons were performed using one-way ANOVA followed by Dunnett's multiple comparison test, with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

CELL VIABILITY ANALYSIS

A concentration of 10, 20, 40 µM telmisartan significantly ($p<0.0001$) reduced cell viability compared to the control group, whereas the other tested concentrations showed no significant effect. Sorafenib did not reduce it significantly at 1 µM concentration, and 0.25 µM was selected for subsequent experiments for both drugs. The cell viability results of Telmisartan and Sorafenib are presented in Figures 1 and 2, respectively.

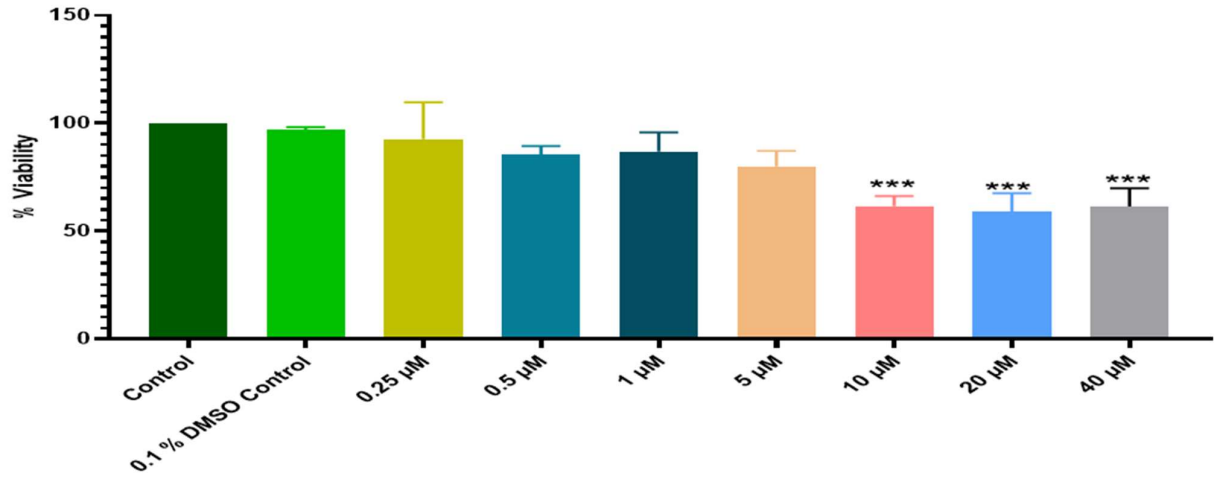

Figure 1. Effect of Telmisartan on H9C2 (2-1) cell viability as assessed by the MTT assay. Data are expressed as mean ± SEM (n = 3).

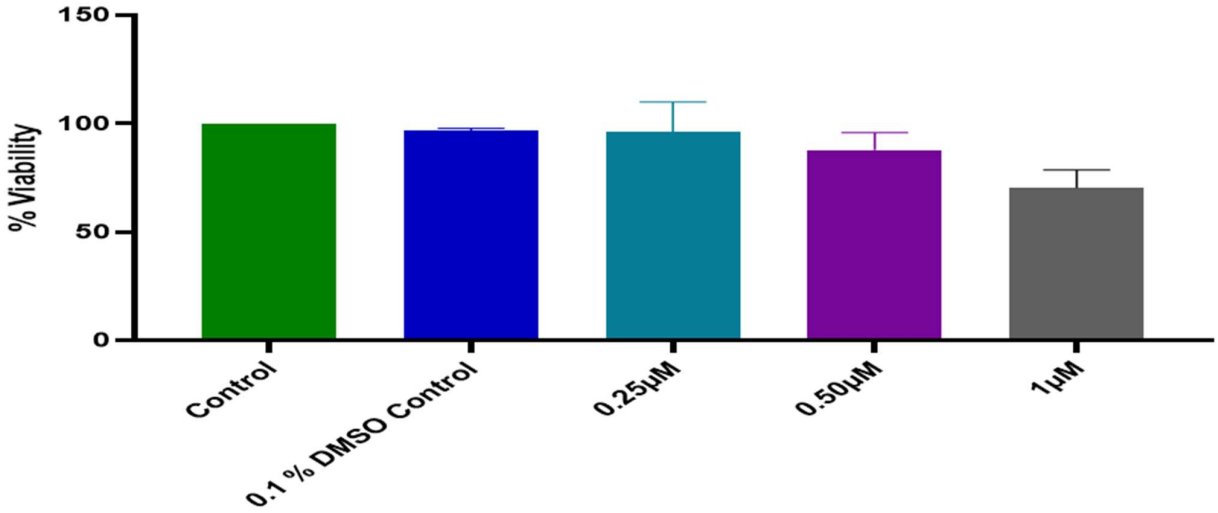

Figure 2. Effect of Sorafenib on H9C2 (2-1) cell viability as assessed by the MTT assay.

Data are expressed as mean ± SEM (n = 3).

EFFECT OF TELMISARTAN AND SORAFENIB ON SALT-INDUCED CARDIAC CELL HYPERTROPHY

Salt exposure successfully induced cardiac hypertrophy in the H9C2 (2-1) cell line, as evidenced by a significant increase in cell size in the disease control (DC) group compared with the normal control (NC) group (####, p = 0.0001). Treatment with Telmisartan at concentrations of 0.25 µM (T1, ***, p = 0.0001), 0.20 µM (T2, **, p = 0.001), and 0.15 µM (T3, *, p < 0.05) resulted in a significant, dose-dependent reduction in cell area relative to the DC group. In addition, Sorafenib at 0.25 µM (S1) also significantly decreased cell size compared with the DC group (*, p < 0.05). The results are illustrated in Figures 3, and 4, respectively.

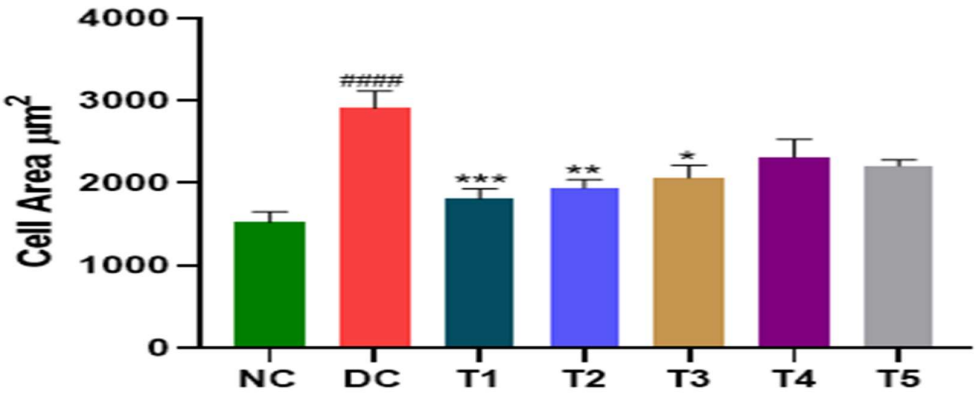

Figure 3. Effect of different concentrations of Telmisartan on cell surface area in salt-induced hypertrophic H9C2 (2-1) cells. Data are expressed as mean ± SEM (n = 3).

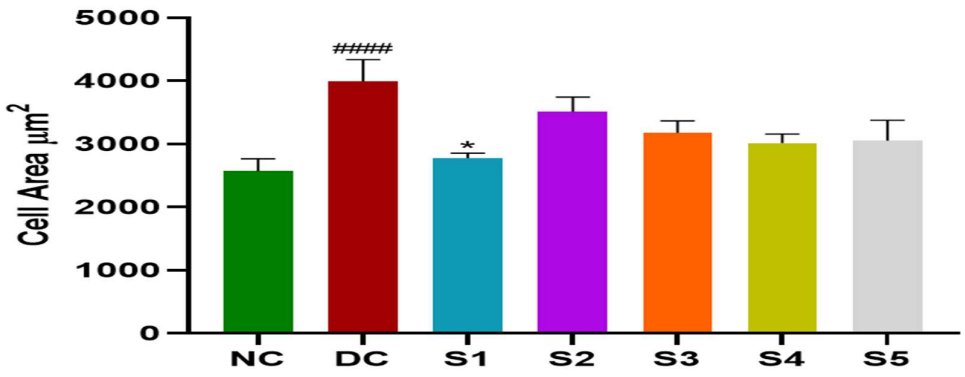

Figure 4. Effect of different concentrations of Sorafenib on cell surface area in salt-induced hypertrophic H9C2 (2-1) cells. Data are expressed as mean ± SEM (n = 3).

EFFECT OF DIFFERENT CONCENTRATIONS OF TELMISARTAN AND SORAFENIB ON A-SMA LEVEL

Salt exposure led to a significant increase in α-smooth muscle actin (α-SMA) levels in the disease control (DC) group of H9C2 (2-1) cells compared with the normal control (NC) group (#, $p < 0.05$). However, treatment with different concentrations of Telmisartan and Sorafenib did not produce any statistically significant changes in α-SMA levels when compared with the DC group. The results are presented in Figures 5 and 6, respectively.

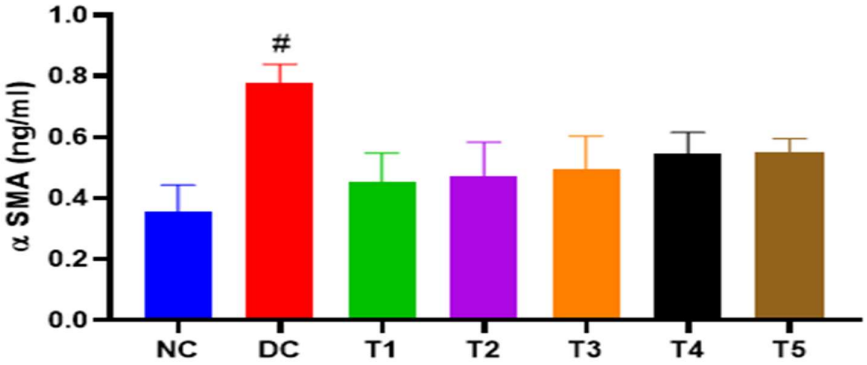

Figure 5. Effect of different concentrations of Telmisartan on α-smooth muscle actin (α-SMA) levels in salt-induced hypertrophic H9C2 (2-1) cells. Data are expressed as mean ± SEM (n = 3).

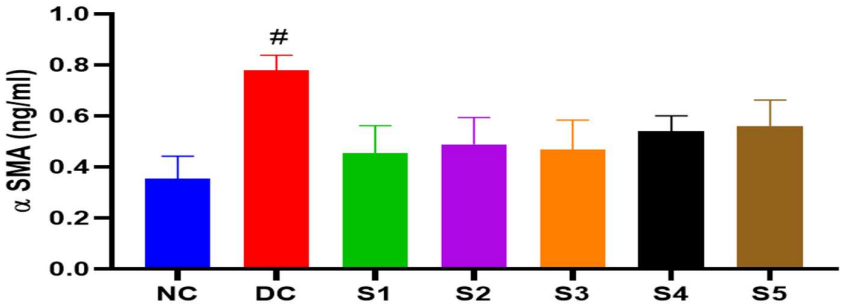

Figure 6. Effect of different concentrations of Sorafenib on α-smooth muscle actin (α-SMA) levels in salt-induced hypertrophic H9C2 (2-1) cells. Data are expressed as mean ± SEM (n = 3).

CONCLUSION

In summary, the present study establishes a reliable in vitro high-salt model of cardiac hypertrophy using H9C2 (2-1) cells. Salt exposure induced significant cardiomyocyte hypertrophy and increased α-SMA level, reflecting pathological remodeling. Treatment with Telmisartan significantly and dose-dependently reduced salt-induced cellular hypertrophy, while Sorafenib also demonstrated a significant anti-hypertrophic effect at a non-cytotoxic concentration. However, neither drug significantly attenuated α-SMA levels, indicating that short-term treatment may be insufficient to reverse fibrotic signaling in this model. Overall, these findings suggest that Telmisartan, similar to Sorafenib, can mitigate salt-induced cardiac hypertrophy but not fibrosis in this study. Further Invivo validation is required.

DECLARATIONS

Ethics approval and consent to participate: Not Applicable

Consent for publication: Not Applicable

Availability of data and material: Not Applicable

Funding: Not Applicable

Authors' contributions: AKP designed, conceived, collected data, and wrote the manuscript. GS supervised the work and reviewed the manuscript

Authors' information (optional):

ORCID ID of AKP: 0000-0001-6068-0616

ORCID ID of GS: 0000-0003-0769-3914

REFERENCE

Dostal DE (2000) The cardiac renin–angiotensin system: novel signaling mechanisms related to cardiac growth and function. Regul Pept 91:1–11. https://doi.org/10.1016/S0167-0115(99)00123-8

Hilfiker-Kleiner D, Landmesser U, Drexler H (2006) Molecular Mechanisms in Heart Failure. J Am Coll Cardiol 48:A56–A66. https://doi.org/10.1016/j.jacc.2006.07.007

Holm Nielsen S, Willumsen N, Leeming DJ, et al (2019) Serological Assessment of Activated Fibroblasts by alpha-Smooth Muscle Actin (α-SMA): A Noninvasive Biomarker of Activated Fibroblasts in Lung Disorders. Transl Oncol 12:368–374. https://doi.org/10.1016/j.tranon.2018.11.004

Kong P, Christia P, Frangogiannis NG (2014) The pathogenesis of cardiac fibrosis. Cellular and Molecular Life Sciences 71:549–574. https://doi.org/10.1007/s00018-013-1349-6

Li X, Lan Y, Wang Y, et al (2017) Telmisartan suppresses cardiac hypertrophy by inhibiting cardiomyocyte apoptosis via the NFAT/ANP/BNP signaling pathway. Mol Med Rep 15:2574–2582. https://doi.org/10.3892/mmr.2017.6318

Pap D, Pajtók C, Veres-Székely A, et al (2023) High Salt Promotes Inflammatory and Fibrotic Response in Peritoneal Cells. Int J Mol Sci 24:13765. https://doi.org/10.3390/ijms241813765

Raut H, Jadhav C, Shetty K, et al (2022) Sorafenib tosylate novel drug delivery systems: Implications of nanotechnology in both approved and unapproved indications. OpenNano 8:100103. https://doi.org/10.1016/j.onano.2022.100103

Vukelic S, Griendling KK (2014) Angiotensin II, From Vasoconstrictor to Growth Factor. Circ Res 114:754–757. https://doi.org/10.1161/CIRCRESAHA.114.303045

Wenzel UO, Bode M, Kurts C, Ehmke H (2019) Salt, inflammation, IL-17 and hypertension. Br J Pharmacol 176:1853–1863. https://doi.org/10.1111/bph.14359

Zhang L, Joseph L, Joseph J (2019) In Vivo and In Vitro Effects of Vasopressin V2 Receptor Antagonism on Myocardial Fibrosis in Rats. Am J Med Sci 357:151–159. https://doi.org/10.1016/j.amjms.2018.11.010