Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

THRAATCHATHI CHOORANAM, A POLYHERBAL SIDDHA FORMULATION PROTECTS AGAINST ISOPROTERENOL INDUCED BIOCHEMICAL ALTERATIONS IN RATS

RAMAKRISHNAN GANAPATHY[1,2], GAYATHRI VEERARAGHAVAN[1], GIRISH RAMESH[1], GAYATHRI KARANAM[1], SIVARAMAN GURUSAMY[4] AND SARAVANA BABU CHIDAMBARAM[1,3*]

[1]Centre for Toxicology and Developmental Research, Sri Ramachandra University, Porur, Chennai-600 116, Tamil Nadu, India
[2]Department of Biochemistry, PRIST University, Vallam, Thanjavur-613 403, Tamil Nadu, India
[3]Dept of Pharmacology, JSS College of Pharmacy, Mysuru-570015, Karnataka, India
[4]Arogya Health Care Pvt Ltd, Chennai -600 050,Tamil Nadu, India.

ABSTRACT

Current therapeutic approaches in the management of cardiovascular diseases like acute myocardial infarction, cardiac reperfusion injury and hypertension produce side effects on myocardium on long term treatment. The present study was undertaken to investigate the cardioprotective effects of Thraatchathi chooranam, a polyherbal siddha formulation against Isoproterenol (ISO) induced biochemical alterations in Sprague Dawley rats. Administration of ISO in rats significantly elevated plasma creatine kinase (CK-MB), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST) and lipids such as total cholesterol (TCHO), triglycerides (TG) and low density lipoproteins with a concurrent decrease in high density lipoproteins (HDL) levels in comparison to vehicle treated control rats. Pretreatment with Thraatchathi chooranam (50 & 100 mg/kg, p.o) for a period of 28 days significantly prevented these alterations in ISO challenged rats. Results were comparable with that of the standard anti-oxidant Vit E. These data's provides evidence on the cardioprotective effects of Thraatchathi chooranam, at least, partly through the anti-oxidant chemical principles and mechanism.

KEYWORDS: **Myocardial necrosis, Thraatchathi chooranam, Isoproterenol, Heart Disease, Cardiac biomarkers**

SARAVANA BABU CHIDAMBARAM
Dept of Pharmacology, JSS College of Pharmacy, Mysuru-570015, Karnataka, India

INTRODUCTION

It is well known that activation of sympathetic nervous system by various physiological stimuli releases catecholamines into the blood circulation. Even though initial actions of catecholamines have a significant role in regulating cardiac function, their chronic effects pose more myocardial injury[1]. Exposure of myocardial cells to continuous and high concentration of catecholamines have been reported to deplete high energy phosphates (ATP), in conjunction with a various biochemical and structural alterations leading to cell damage, which is a prologue to myocardial necrosis[2, 3]. Isoproterenol (ISO) model of myocardial necrosis is used as a classical method to induce infarct-like lesions in the rodents and other species [4]. ISO is a synthetic catecholamine, which stimulates both β1- and β2-adrenergic receptors and produces an intense increase in inotropic and chronotropic effects and in turn elevation of cardiac biomarkers such as CK -MB, troponin 1 etc. and generation of oxygen radicals[5-6] . These functional alterations are similar to that occurring in patients with myocardial infarction. The current therapeutic approaches are designed to reduce infarct size and preserve the myocardium from ischemia/reperfusion injury, but their delayed effects on the myocardium are deleterious. Therefore, newer approaches are desperately needed in order to minimize them. Natural resources such as fruits, extracts, polyherbal and herbomineral formulations, have been used to treat various metabolic diseases since ancient ages. Several studies have been reported that herbal remedies and their bioactive compounds are being useful for control of ischemia induced necrosis and associated pathological changes[7]. Siddha system of medicine is one of the oldest traditional medicinal systems in India, which has been widely practiced in Tamil Nadu. Siddha medicine preparation and their therapeutics depend on natural resources[8], to revitalize the integral part of organs, and thereby improving the quality of human life. Siddha system of medicine has gained constructive attention and its therapeutic interventions for different metabolic disorders and diseases, including heart disease in recent years. Although the Siddha literature describes the information about therapeutic activities of many medicinal plants or polyherbal formulations, there is paucity on the standardization, quality control and scientific evidences through reverse pharmacological screening. It may be explored with an advanced scientific approach for better leads in the healthcare[9] and also improving the scientific credibility of Siddha system of medicine. Thraatchathi chooranam (TC), a siddha polyherbal formulation has been traditionally used as a cardiac tonic and also as adjuvant in the management of diabetes mellitus. Recently, we reported the presence of polyphenols such as Gallic acid, Ellagic acid, Narginine, Quercetin, Galangin in Thraatchathi chooranam[10]. These phenolic principles are shown to play a key role as antioxidant and cardio protective agents[11]. The present investigation is undertaken to evaluate the ability of TC to alleviate the biochemical changes induced by the ISO administration in rats.

MATERIAL & METHODS

1. Drugs and Chemicals

Thraatchathi chooranam was procured commercially from a Siddha clinic M/s. Arogya Healthcare Pvt. Ltd., Chennai. Alpha tocopherol and Isoproterenol were purchased from Sigma Chemical Co., St. Louis, MO, USA. Tri-sodium citrate was purchased from MERCK. Biochemical kits such as Creatine kinase-MB (CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total Cholesterol (TCHO), Trigercerides (TG), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) were purchased from Accurex Biomedical Pvt Ltd, Thane, India.

2. Animals

Adult Male Sprague Dawley rats (150-200g) were used for the study. The animals were housed in an air–conditioned room at 23 ± 2 °C (RH: 30 - 70%, 12h-12h day light cycle). They were fed with standard laboratory diet (Amrut Laboratory Animal Feed, Pune) and water *ad libitum*. The animals were procured from Biogen, Bangalore. This study was approved by the Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai (IAEC-XXXVI/SRU/336/2013).

3. Experimental Design

Five days after acclimatization, the rats were divided into 6 groups with 6 in each and treatment was carried out as mentioned below.

Group I : Normal control: 0.5% CMC + saline Subcutaneous (s.c)

Group II: Positive control: 0.5%CMC + ISO (120 mg/kg, s.c, twice at 48 h interval)

Group III: Standard: Vit E (100 mg/kg/day, p.o+ ISO (120 mg/kg, sc twice at 48 h interval)

Group IV: Low Dose: TC (50 mg/kg/day, p.o + ISO (120 mg/kg, sc twice at 48 h interval)

Group V: High Dose: TC (100 mg/kg/day, p.o+ ISO (120mg/kg, sc twice at 48 h interval)

Group VI: Drug control- TC (100 mg/kg/day, p.o)

4. Induction of Myocardial necrosis

Experimental animals were pre-treated once a day for a period of 28 days with respective drugs viz: Vehicle / Vit E / Thraatchathi chooranam. On day 26th and 28th, myocardial necrosis was induced by subcutaneous injection of two doses of ISO at an interval of 48 hours. On day 29, animals were anesthetized, blood was collected through retro orbital puncture and plasma was separated using Tri-sodium citrate as anti-coagulant and used for the biochemical estimation.

5. Biochemical Assay - Estimation of Plasma Biomarker levels

Biochemical assays were performed as per the Accurex kit insert using semi-automated biochemical analyser (Star21Plus, India).

6. Statistical Analysis

Data were expressed as mean ± standard error mean (SEM). Mean differences between the groups were analyzed by one way ANOVA followed by Tukey's multiple comparison as posthoc test. P value ≤ 0.05 was

considered as statistically significant. Statistical analysis was performed using GraphPad Prism, 5.0 San Diego, USA.

RESULTS

Current investigation was undertaken to unravel the cardioprotective effects of Thraatchathi Chooranam on biochemical alterations following isoproterenol administration in rats. The effect of the Thraatchathi Chooranam on body weight was recorded once a week for 28 days. On day 28, ISO group showed significant ($p<0.01$) decrease in body weight (Fig 1) when compared to control group, whereas animals pretreated with Thraatchathi Chooranam (50 &100 mg/kg) significantly improved the body weight ($p<0.01$) when compared to ISO rats. A significant increase ($p<0.05$) in heart weight (Table – 1) was observed in the ISO group in comparison to control group, whilst pretreatment with TC ameliorated the ISO induced changes in heart weight. Results of TC were comparable with Vit E.

Biochemical Assays

TC decreased plasma CK-MB and LDH levels in ISO challenged rats

ISO treated group showed significant ($p < 0.01$) increase in the levels of CK-MB and LDH compared to control group. Pretreatment with Thraatchathi chooranam prevented the ISO induced elevation of these parameters in plasma in a dose dependent manner ($p < 0.01$) when compared to ISO induced rats (Fig 2a-2b). Drug control group showed normal levels of cardiac biomarker in plasma.

TC restores AST and ALT levels in ISO challenged rats

Fig 3a and 3b show the effects of TC on liver function in ISO challenged rats. Rats treated with ISO showed a significant ($p<0.01$) increase in the levels of AST and ALT as compared to the control group. Pretreatment with TC for 28 days and challenged with ISO showed a significant ($p <0.01$) reduction in AST and ALT levels when compared to ISO treated rats. Results were comparable to Vit E. The drug control group showed normal levels of AST and ALT markers in plasma.

Estimation of Plasma Lipid profile

Effect of TC on lipid profiles in ISO induced rats are illustrated in Fig 4a to 4d. ISO administered rats showed significant ($p < 0.01$) increase in plasma cholesterol, TG, LDL, and decrease in HDL levels when compared to the vehicle treated control group. Rats pretreated with Thraatchathi chooranam showed dose dependent decrease in TCHO, TG and LDL with significant ($p<0.01$) increase in HDL levels compared to ISO treated rats. Results are comparable with standard anti-oxidant Vit-E. Drug control group showed normal lipid profile.

DISCUSSION

Isoproterenol (ISO) induced myocardial necrosis is a standard model to study the cardioprotective effects of newer therapeutic agents. In the present study, the cardioprotective effects Thraatchathi chooranam (TC) was studied in rats intoxicated with Isoproterenol[12]. The effects of TC were compared with Vit E, a major antioxidant in lipid phases that can protect myocardium against oxidative stress. The body weight was monitored once a week for a period of 28 days in order to evaluate the drug effects on normal physiology. A significant reduction in body weight and increase in the heart weight were observed in ISO treated rats. ISO intoxication was shown to produce cardiac hypertrophy[13], a similar effect was observed in the present study. Pretreatment with TC (50 & 100 mg/kg) prevented the cardiac hypertrophy probably through the inhibition of edema formation and cytokines generation[14], which could be attributed by the presence of high phenolic principles. Isoproterenol induced cardiotoxicity intensively damage the cardiac cells. As a result of this, transient increases in cytosolic enzymes were released into the blood stream, which is an indicator of myocardial injury[15]. The amount of these enzymes present in the plasma reflects the functional changes in cell membrane integrity and/or permeability[16]. In our study, ISO administered group showed a significant elevation in the levels of cardiac enzymes CK-MB and LDH in plasma. These elevations of cardiac specific enzymes in plasma indicate severity of ISO induced myocardial tissue damage, thus confirming the myocardial injury and this observation are in harmony with earlier reports[15, 17-18]. Pretreatment with TC (50, 100 mg/kg) significantly restored the cardiac specific enzymes in plasma when compared to only ISO treated rats and thus indicating the membrane stabilizing effect of TC. Earlier it has been reported that polyphenols are responsible for maintaining normal structural and functional integrity of cardiac myocytes[19]. So in our study, the high polyphenolic content in TC would have played a key role in maintaining the functional integrity to counteract ISO induced in myocardial membrane damage. High dose of Isoproterenol cause myocardial injury can be attributed to leakage of enzymes (AST and ALT) from heart tissue into the blood stream[20]. In our study, we observed significant elevation of AST and ALT enzymes in plasma due to the damaged structural integrity of the heart by ISO which is in consistent with earlier reports[21, 13]. Pretreatment with TC (50 & 100 mg/kg) and Vit E might have minimized the effect of ISO and would have prevented the leakage, thereby maintaining the structural integrity of membrane. Lipids are essential for energy storage, signaling and serves as structural components of biological membranes. Alteration in lipid metabolism might lead to serious consequences and is considered to accelerate the structural and functional changes of myocardium by modifying the composition and stability of cellular membranes may contribute to the cell death[22]. Previously it was reported that activation of sympathetic nervous system releases catecholamines at high concentration causing distortion of cardiac lipid metabolism and decompensate cardiac dysfunction[23-24]. In our study, we observed a significant increase in the levels of triglycerides (TG), total cholesterol (TCHO) and low density lipoprotein (LDL) in ISO treated rats with concomitant decrease in the level of high density lipoprotein (HDL) in the plasma when compared with vehicle treated group which are consistent with the earlier findings[25-27]. These significant changes occur in lipid composition levels in plasma due to enhance lipid

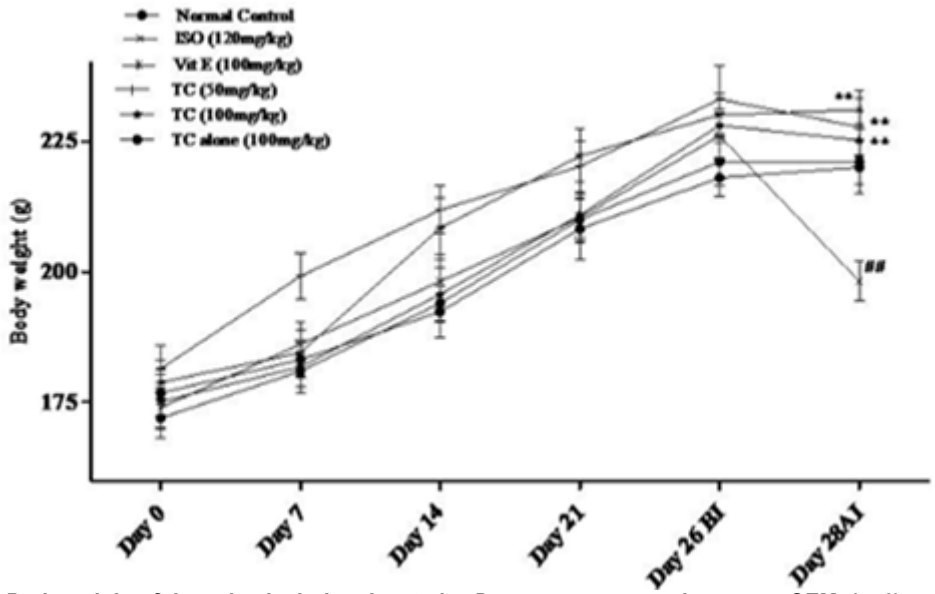
biosynthesis by cardiac cyclic AMP[28] or increased hepatic cholesterol biosynthesis. Numerous studies have reported beneficial effects of dietary antioxidants, mainly polyphenols in heart diseases. It is has been reported that polyphenols have potent hypolipidemic effect[29,30]. Pretreatment with TC (50, 100mg/kg) showed significantly lowered TG, TCHO and LDL levels in plasma, and increased HDL in a dose-dependent manner when compared with ISO treated rats. The hypolipidemic effects of TC might be due to inhibition of cholesterol biosynthesis and increase the uptake of LDL from the blood stream by the liver, probably due to the presence of phenolic principles. A large number of studies have shown that ingestion of polyphenol rich foods attenuate hypercholesterolemia[31]. Several ingredients of Tharaachathi chooranam like *Vitis vinifera, Elettaria cardamomum, Cuminum cyminum, Emblica officinalis, Terminalia chebula, Cyperus rotundus, Zingiber officinale [dried], Glycyrrizha glabra, Nelumbo nucifera, Piper longum* are known to have hypolipidemic effects. From these data it is clear that the protective effects observed with TC against ISO induced biochemical changes might be due to the presence of high polyphenols content.

Table 1
Effect of TC on Heart weight in experimental rats

Group	Treatment	Heart (g)
Normal Control	0.5%CMC, p.o + saline s.c	0.97±0.08
ISO	0.5% CMC, p.o + ISO (120 mg/kg, s.c)	1.30±0.10#
Vit E	100 mg/kg/day, p.o+ ISO (120 mg/kg, sc)	0.96±0.06*
TC	50 mg/kg/day, p.o + ISO (120 mg/kg, sc)	1.10±0.07
	100 mg/kg/day, p.o + ISO (120 mg/kg, sc)	1.02±0.08
TC alone	100 mg/kg/day, p.o	0.95±0.05

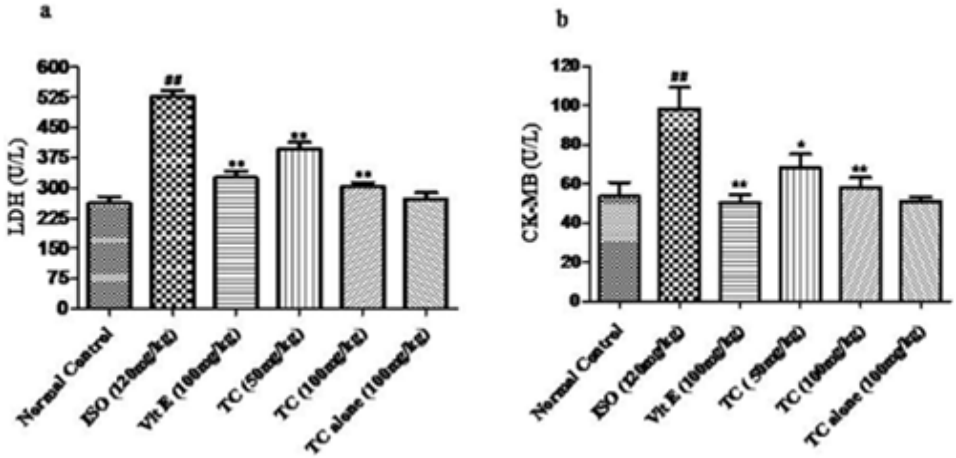
Note: The results are expressed in mean ± SEM (n =6); and analysed by one way ANOVA followed by Tukey's multiple comparsion as post hoc test. #P < 0.05 and ## P < 0.01 vs. Normal control and *P < 0.05 and ** P < 0.01 vs. ISO

Figure 1



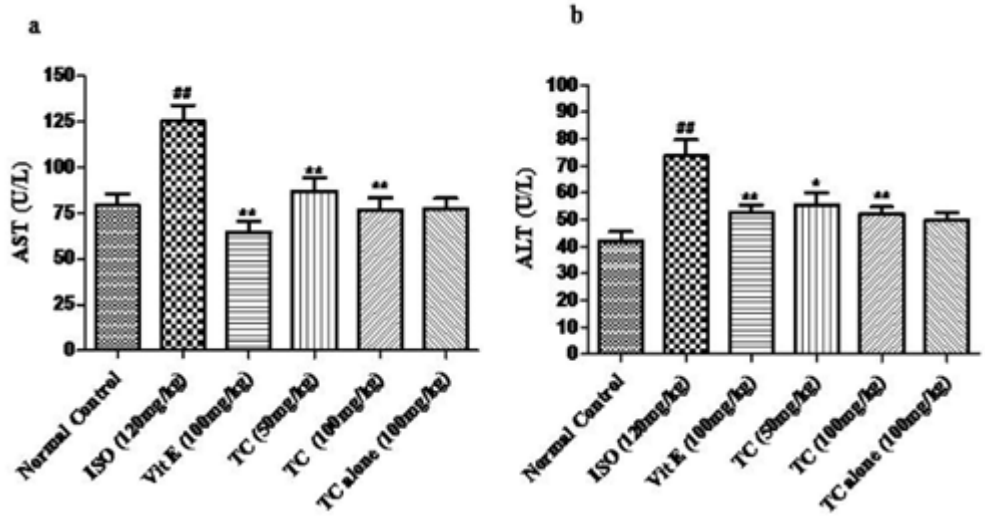
The effect of TC on the Body weight of the animals during the study . Data were expressed as mean±SEM; (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparsion as post hoc test. #P < 0.05 and ## P < 0.01 vs. Normal control and *P < 0.05 and ** P < 0.01 vs. ISO

Figure 2



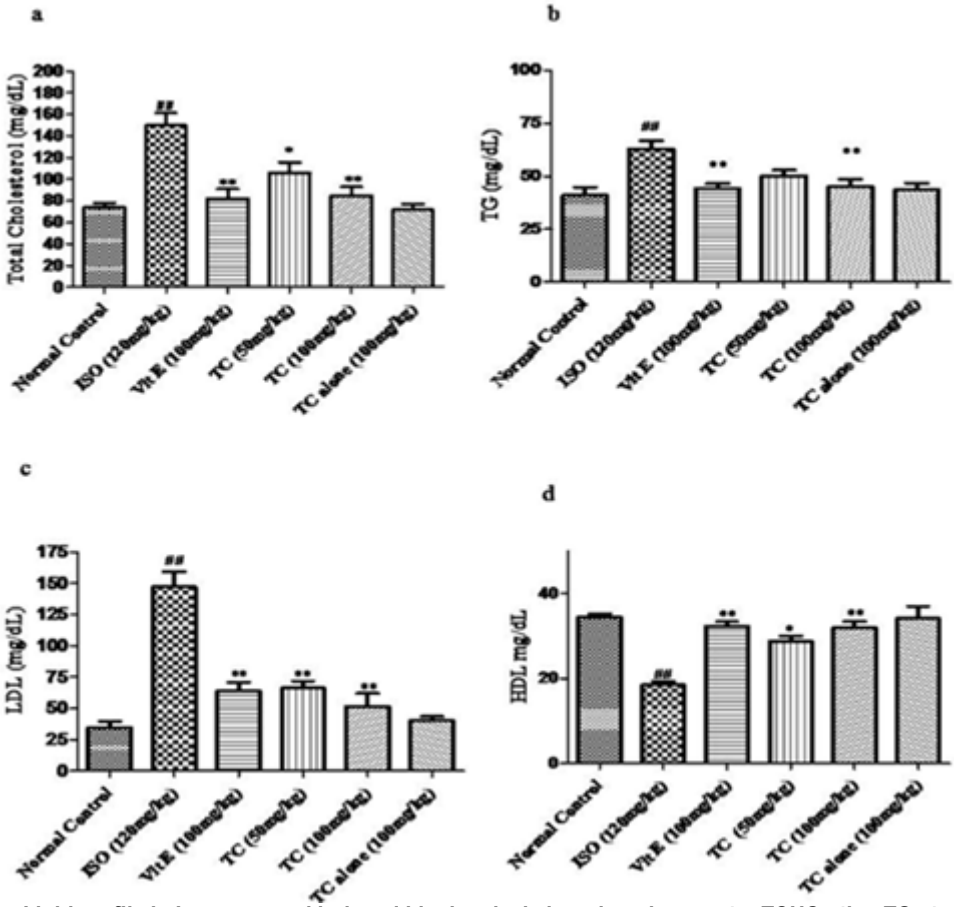
Effects of TC on plasma cardiac biomarkers level in Isoproterenol induced biochemical alterations in rats. 2a – CKMB; 2b – LDH.Data were expressed as mean ± SEM; (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparsion as post hoc test. #P < 0.05 and ## P < 0.01 vs. Normal control or *P < 0.05 and ** P < 0.01 vs. ISO

Figure 3



***Effects of TC on plasma cardiac biomarkers level in Isoproterenol induced biochemical alterations in rats. 3a- AST; 3b – ALT. Data were expressed as mean ± SEM; (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparsion as post hoc test. #P < 0.05 and ## P < 0.01 vs. Normal control and *P < 0.05 and ** P < 0.01 vs. ISO*:**

Figure 4



Effects of TC on plasma Lipid profile in Isoproterenol induced biochemical alterations in rats. 4a- TCHO; 4b – TG; 4c –LDL; 4d – HDL. Data were expressed as mean ± SEM; (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparsion as post hoc test. $^{\#}P < 0.05$ and $^{\#\#} P < 0.01$ vs. Normal control and $^{*}P < 0.05$ and $^{**} P < 0.01$ vs. ISO

CONCLUSION

Summarily, it can be concluded that Thraatchathi chooranam, possess significant anti-oxidant effects against catecholamines induced free radicals and hence can be considered for further research activities.

ACKNOWLEDGEMENT

Authors acknowledge the CEFT staff for the support render during experimentation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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