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Evaluation of the Effect of Stone Herbal Mixture Drink on Cardiac Markers (Troponin I, C & T)

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Abstract

Cardiovascular diseases (CVDs) continue to be a major global health concern, contributing to a significant proportion of morbidity and mortality worldwide. As our understanding of the intricate mechanisms underlying cardiac health and disease evolves, researchers have turned their attention to exploring potential alternative or complementary therapies. Herbal remedies have been a subject of increasing interest due to their historical use and potential to influence physiological processes. Troponins, specifically Troponin I (Ti) and Troponin T (Tt) are critical cardiac markers widely recognized for their diagnostic significance in assessing myocardial injury. Elevated levels of these markers indicate cardiac muscle damage, often associated with conditions like myocardial infarction and heart failure. Investigating interventions that could influence these cardiac markers presents an avenue for exploring potential therapeutic strategies and preventive measures against CVDs. This study was an experimental study comprising of total of thirty-six (36) Wistar rats were divided into five (6) groups of six (6) animals each. The six groups were Group 1, control group fed with top feed and water only, Group 2 standard drug (Aspirin 500mg/kg body weight) + 0.30mls of stone herbal mixture, Group 3 with 0.30ml of stone herbal mixture, Group 4 with 0.25ml of stone herbal mixture, Group 5 with 0.15ml of stone herbal mixture and Group 6 with 0.10ml of stone herbal mixture. The treatment was given daily and lasted for 21 days. Blood was collected into Lithium Heparin anticoagulant bottle from brachiocephalic artery of each rat and the sample analyzed for cardiac parameters. Group 3,4,5 & 6 which contains 1000mg, 850mg, 650mg, and 500mg of stone herbal mixture showed significant increase of this cardiac marker troponin I statistically ($p<0.01$) with percentage of 21 %, 27%, 64 % and 77 % respectively when compared to the control group one. Also, group two, administered with 1000mg of the stone herbal mixture and 500mg per kg body weight of aspirin totally reversed toxicity compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this cardiac marker troponin C ($p<0.05$) with percentage of 23%, 29%, 70% and 111% respectively when compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this cardiac marker troponin T ($p<0.05$) with percentage of 26%, 29%, 64% and 121 % respectively when compared to the control group one. In conclusion, this study underscores the need for caution when using Stone Herbal Mixture, as higher doses observed from the increasing volume administered may pose a risk to heart health. The cardioprotective effect of aspirin observed in this study emphasizes the potential benefits of combining herbal remedies with established medications for cardiovascular health.

Keywords: Stone Herbal, Mixture, Cardiac Markers, Troponin

Introduction

The Stone Herbal Mixture (SHM), a traditional herbal formulation, has gained attention for its purported cardio-protective properties. Ancient herbal knowledge suggests that SHM may possess bioactive compounds capable of modulating cardiovascular health. While anecdotal evidence points to the potential

benefits of SHM, rigorous scientific investigation is required to establish its efficacy and elucidate the mechanisms involved (Iyevhobu *et al.*, 2022).

Also, herbal remedies often embody a holistic approach to healthcare, addressing multiple aspects of well-being (Iyevhobu *et al.*, 2022). Exploring the potential cardio-protective effects of SHM aligns with the growing interest in holistic and integrative approaches to managing and preventing cardiovascular diseases. Despite advancements in medical treatments, some individuals may seek alternatives due to concerns about side effects or limited treatment efficacy. If SHM demonstrates positive effects on cardiac markers, it could provide an additional option for individuals looking to manage their cardiovascular health.

Cardiovascular diseases (CVDs) continue to be a major global health concern, contributing to a significant proportion of morbidity and mortality worldwide. As our understanding of the intricate mechanisms underlying cardiac health and disease evolves, researchers have turned their attention to exploring potential alternative or complementary therapies. Herbal remedies have been a subject of increasing interest due to their historical use and potential to influence physiological processes (Iyevhobu *et al.*, 2022). Cardiovascular diseases (CVDs) continue to pose a substantial burden on global public health, accounting for a significant number of morbidity and mortality cases. Cardiovascular diseases are the leading cause of death worldwide except Africa. Together CVD resulted in 17.9 million deaths (32.1%) in 2015, up from 12.3 million (25.8%) in 1990. Deaths, at a given age, from CVD are more common and have been increasing in much of the developing world while rates have declined in most of the developed world since the 1970s.

Cardiovascular diseases (CVDs) remain a leading cause of mortality globally. Investigating potential therapeutic interventions, such as herbal remedies, could contribute to addressing this significant public health issue (Iyevhobu *et al.*, 2022). Understanding the impact of Stone Herbal Mixture (SHM) on cardiac markers may provide insights into its potential as an adjunctive or preventive treatment for cardiovascular health. Traditional medicine has a rich history of using herbal formulations for various health conditions (Iyevhobu *et al.*, 2022). By scientifically investigating the effects of SHM, we can bridge the gap between traditional knowledge and modern medical research, potentially unlocking new avenues for drug development or complementary therapies.

Troponins, specifically Troponin I (Ti) and Troponin T (Tt) are critical cardiac markers widely recognized for their diagnostic significance in assessing myocardial injury. Elevated levels of these markers indicate cardiac muscle damage, often associated with conditions like myocardial infarction and heart failure. Investigating interventions that could influence these cardiac markers presents an avenue for exploring potential therapeutic strategies and preventive measures against CVDs. Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females (Lippi *et al.,* 2020). In the United States 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD (Moran *et al.,* 2014).

Despite advancements in medical interventions, the prevalence of CVDs remains a pressing concern, prompting the exploration of alternative and complementary therapies. Herbal remedies have gained attention for their potential to influence physiological processes and offer cardio-protective effects (Iyevhobu *et al.*, 2022). In this context, the research problem centers on the need to systematically investigate the potential impact of Stone Herbal Mixture (SHM) on cardiac markers in male albino Wistar rats.

Research conducted on animal models offers a controlled environment to study the effects of interventions on specific physiological processes. Male albino Wistar rats have been commonly employed as experimental subjects due to their genetic homogeneity and physiological similarity to humans. Utilizing this animal model provides an opportunity to explore the impact of SHM on cardiac markers in a controlled setting, enabling the assessment of potential cardio-protective effects and mechanisms of action.

While SHM has been traditionally used for its purported cardio-protective properties, a dearth of rigorous scientific investigations hampers the establishment of its efficacy and mechanisms of action. The absence of empirical data undermines informed decision-making by healthcare practitioners and individuals seeking alternative cardiovascular interventions. Despite the historical use of herbal remedies for heart health, the specific effects of SHM on cardiac markers, such as Troponin I, C, T remain largely unexplored (Iyevhobu *et al.*, 2022). A comprehensive examination of these cardiac markers following SHM administration is lacking, leaving a significant gap in our understanding of the potential therapeutic benefits of this herbal formulation.

Also, in a healthcare landscape where patients and practitioners seek effective and evidence-based treatment options, the absence of scientific inquiry into SHM's effects on cardiac markers limits the availability of alternative interventions. There is a demand for credible information on whether SHM holds promise as a preventive or complementary treatment strategy for cardiovascular diseases.

Utilizing male albino Wistar rats as an animal model allows for controlled experimentation and observation of physiological changes. This study could lay the foundation for further research, potentially leading to clinical trials or investigations into the mechanisms of action underlying SHM's effects. Herbal remedies often offer a more affordable option for healthcare, particularly in regions with limited access to advanced medical treatments (Iyevhobu *et al.*, 2022). Understanding the potential benefits of SHM could contribute to cost-effective preventive strategies.

This study aims to bridge the gap between traditional herbal knowledge and modern scientific exploration by investigating the effect of Stone Herbal Mixture on cardiac markers (Ti, Tc and Tt) in male albino Wistar rats. By conducting a comprehensive evaluation of these cardiac markers following SHM administration, we seek to contribute to the growing body of knowledge on herbal interventions for cardiovascular health. Ultimately, this research could potentially uncover novel therapeutic avenues and provide insights into the development of preventive strategies against cardiovascular diseases.

As we delve into this investigation, it is important to acknowledge the interdisciplinary nature of this study, drawing from fields such as pharmacology, cardiology, and traditional medicine. By combining ancient wisdom with contemporary scientific methodologies, we aspire to shed light on the potential benefits of SHM and contribute to the advancement of cardiovascular health research.

Materials and Method

Materials

Materials used in this study include; Cotton wool, needle and syringe, plain bottle, Lithium heparin, methylated spirit, Gloves, micropipette, Tourniquet, automatic micropipette, pipette tips, spectrophotometer, spectrophotometric cuvette, water bathe, vortex mixer, disposable test tubes, and stone herbal mixture.

Study Design

This study was an experimental study comprising of total of 36 (36) Wistar rats divided into five (5) groups of six (6) animals each. The six groups were Group 1, control group fed with top feed and water only, Group 2 standard drug (Aspirin 500mg/kg body weight) + 0.30mls of stone herbal mixture Group 3 with 0.30ml of stone herbal mixture, Group 4 with 0.25ml of stone herbal mixture, Group 5 with 0.15ml of stone herbal mixture. The treatment was given daily and lasted for 21 days.

Study Site

This study was carried out at the animal house, Department of Medical Laboratory Science, Lead City University Ibadan-Oyo state.

Procurement of *Stone herbal mixture*

Stone Herbal mixture state was purchased from market in Ibadan, Oyo State, Nigeria.

Administration of Stone herbal mixture

The herbal mixture extract was administered with the aid of oral cannula, once daily to the appropriate group for the time specified for each group.

Animal Sacrifice and Sample Collection
At the end of the experiment, blood was collected into Lithium Haparin anticoagulant bottle from brachiocephalic artery of each rat. The rats were immediately ethically sacrificed by cervical dislocation. At the end of the sample collection, the samples were analyzed for cardiac parameters Troponin I (Ti), Troponin C (Tc) and Troponin T (Tt).

Laboratory Analysis
All biochemical assays: Troponin I (Ti), Troponin C (Tc) and Troponin T (Tt) concentrations were carried out using standards kits, methods and an AJ-Semi-auto Biochemical Analyzer following Standard Operative Procedure (SOP).

Statistical Analysis
Data obtained was expressed as mean ± SEM and analysis was done Statistical package for Social Scientists (SPSS version 21.0). Values at $p<0.05$ were considered significant in comparison with appropriate control.

Results

Table 1: Comparison of mean ± SD of Cardiac function parameters in the groups

Cardiac	Group 1 n-6	Group 2 n-6	Group 3 n-6	Group 4 n-6	Group 5 n-6	Group 6 n-6	NR
Troponin I (Ti)	3.7±1.6	4.8±1.5	18.0±1.4	13.7±1.9	5.8±1.5	4.8±1.5	0-14
Troponin C (Tc)	4.2±1.5	7.2±1.5	18.7±2.1	14.5±1.0	6.0±1.4	3.8±1.2	0-14
Troponin T (Tt)	4.0±1.4	7.2±2.3	15.5±3.6	14.0±1.4	6.3±1.2	3.3±1.2	0-14

Table 2: Comparison of Cardiac function parameters

Cardiac Parameters	Group	Mean Difference	t	P-values
Troponin I (Ti)	Group 2	-1.16667	-2.907	.034
	Group 3	-14.33333	-11.660	.000
	Group 4	-10.00000	-9.393	.000
	Group 5	-2.16667	-4.540	.006
	Group 6	-1.16667	-1.337	.239
Troponin C (Tc)	Group 2	-3.00000	-4.108	.009
	Group 3	-14.50000	-15.145	.000
	Group 4	-10.33333	-11.245	.000
	Group 5	-1.83333	-3.841	.012
	Group 6	.33333	.500	.638
Troponin T (Tt)	Group 2	-3.16667	-3.348	.020
	Group 3	-11.50000	-6.977	.001
	Group 4	-10.00000	-11.677	.000
	Group 5	-2.33333	-2.539	.052
	Group 6	.66667	1.195	.286

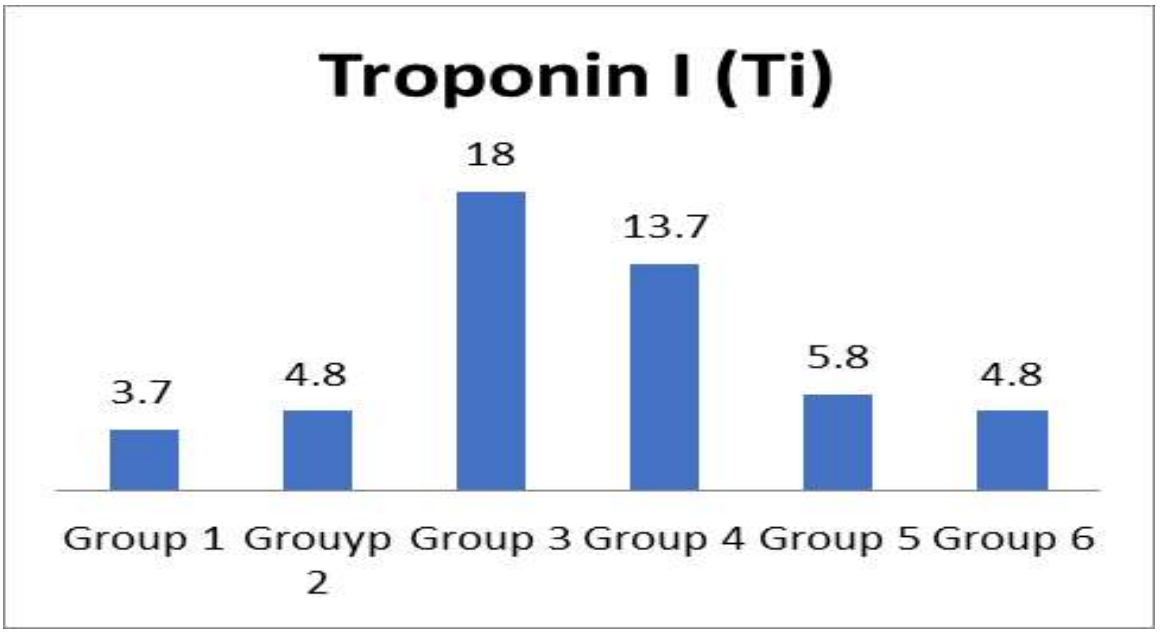

Figure 1: Effect of Stone Herbal Mixture on Troponin I

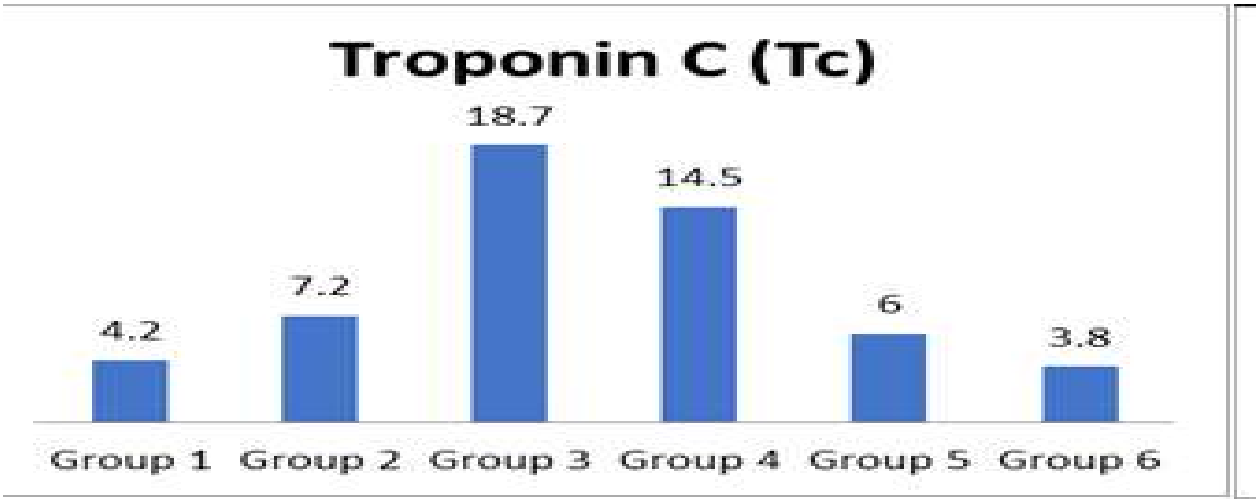

Figure 2: Effect of Stone Herbal Mixture on Troponin C

Figure 3: Effect of Stone Herbal Mixture on Troponin T

Discussion

The investigations into the effect of Stone Herbal Mixture on cardiac markers in male albino Wistar rats yielded crucial insights into the potential impact of this herbal mixture on cardiac health. Findings from the analysis of cardiac markers in Table 1 show significant alterations in the levels of troponin I in some of the treatment groups (Group 3, Group 4, and Group 5). The group two rats that were administered with 1000mg of the stone herbal mixture and 500mg per kg body weight of Aspirin totally reversed toxicity compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this cardiac marker troponin I ($p<0.01$) with percentage of 21 %, 27%, 64 % and 77 % respectively when compared to the control group one.

Table 2 revealed that in a dose dependent manner troponin C was significant in some of the treatment groups (Group 3, Group 4, and Group 5). Group two rats administered with 1000mg of the stone herbal mixture and 500mg per kg body weight of aspirin totally reversed toxicity compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this cardiac marker troponin C statistically at $P<0.05$ with percentage of 23%, 29%, 70% and 111% respectively when compared to the control group one

Table 3 revealed that in a dose dependent manner troponin T was significant in some of the treatment groups (Group 3, Group 4, and Group 5). Group two rats who were administered with 1000mg of the stone herbal mixture and 500mg per kg body weight of Aspirin totally reversed toxicity compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this cardiac marker troponin T statistically at $P<0.05$ with percentage of 26%, 29%, 64% and 121 % respectively when compared to the control group one

The investigation into the effects of Stone Herbal Mixture on cardiac markers in male albino Wistar rats has yielded crucial insights into the potential impact of this herbal mixture on the heart. Findings from the analysis of the markers in Table 1 indicate a significant elevation in the cardiac markers in Group 3, which received the highest volume of the herbal mixture among the treatment groups. The elevated Troponin I levels in Group 3 indicate potential adverse effects of this herbal mixture. This finding aligns with previous literature that often indicates that herbal remedies may have a narrow therapeutic window, and higher doses could lead to adverse effects (Umaru *et al*., 2020; Okaiyeto & Oguntibeju, 2021). Koss-Mikołajczyk *et al*. (2021) also observed similar increases in Troponin I levels in response to high intake of phytochemicals present in herbal mixtures, attributing this to an inflammatory response triggered by excessive intake. Contrastingly, Chaudhary *et al*. (2021) challenged the exclusive attribution of Troponin I elevations to rich-green herbal mixture emphasizing on their cardioprotective properties. This research emphasizes the influence of genetic predispositions and pre-existing health conditions on Troponin I levels. The consistently elevated Troponin C (Tc) levels in Group 3 warrant exploration. Aimo *et al.* (2020) supports these findings, linking high herbal mixture intake to increased Troponin C levels through a mechanism involving oxidative stress and inflammation. Additionally, further evidence was provided in a cross-sectional study, demonstrating a positive correlation between increased volume of herbal mixture intake and Troponin C levels (Netala *et al*., 2024). The role of dietary modification has also been highlighted in reducing Troponin C levels (Juraschek *et al*., 2021). In contrast, lifestyle factors have been introduced as potential influencers of Troponin C levels, emphasizing the need to consider physical activity and other variables in the interpretation of cardiac biomarker outcomes (Adeoye & Olaniyan, 2024). The significant differences in Troponin T (Tt) levels in Group 3 necessitate a comprehensive discussion. Netala *et al*. (2024) provided support by documenting a correlation between elevated Troponin T levels and excess herbal mixture intake. Their proposed mechanism involves an inflammatory response. Koss-Mikołajczyk *et al*. (2021) further corroborate this association in a randomized controlled trial, demonstrating a dose-dependent increase in Troponin T with higher herbal mixture intake. Additionally, Kumar *et al.*

(2018) present evidence from a cross-cultural study, linking dietary patterns rich in excessive herbal mixtures to elevated Troponin T, highlighting the global relevance of these findings. However, Bt Hj Idrus *et al*. (2020) introduce a broader perspective by suggesting that Troponin T elevation may be influenced by factors unrelated to the herbal mixture intake. Their multifactorial model emphasizes genetic factors and overall cardiovascular health, underscoring the complexity of interpreting Troponin T levels.

Despite the fact that the treatment Group 2 received the same volume of the herbal mixture as Group 3, the findings reveal that the cardiac markers were within the reference range. This is probably due to the aspirin that was administered along with the herbal mixture. Aspirin is a well-known medication that is commonly used to protect against cardiovascular disease, particularly in cases of coronary artery disease and the prevention of heart attacks. It primarily functions as an antiplatelet agent, reducing the risk of blood clot formation by inhibiting platelet aggregation (Cox, 2020; Tscharre *et al*., 2020). When compared with the control, groups 2, 3, and 4 show significant differences in their cardiac markers due to the increased volume of the herbal mixture administered. However, the significant difference in Group 2 is not as strong as that of Groups 3 and 4, indicating the cardioprotective function of aspirin (Dong *et al*., 2022). The significant difference seen in Groups 3 and 4 due to the increased volume of the herbal mixture administered, and the non-significant difference seen in Group 5 suggest a dose-response relationship. In a dose-response relationship, the effect of a treatment or substance increases as the dosage or concentration of the substance increases (Moffett *et al*., 2022).

Conclusion

In conclusion, this study has shown valuable insights into the effects of the *Stone Herbal Mixture* on cardiac markers in male albino Wistar rats. Our result demonstrated that the lowest dose of the stone herbal mixture, which is 500 mg is tolerable with the heart as against other higher doses in this experiment. However, the findings demonstrated the necessity for further investigations, comprehensive safety assessments, clinical trials, and increased regulatory oversight to ensure the safety and efficacy of herbal products like the *Stone Herbal Mixture*. Patient safety and informed decision-making should remain important in the use of herbal remedies for healthcare and wellness. While the potential therapeutic benefits of herbal mixtures are enticing, they must be weighed against the potential risks, as demonstrated in this study, to make informed choices about their usage.

Based on the findings of this study, it is crucial to carefully consider the dosage of Stone Herbal Mixture when using it for therapeutic purposes. The results suggest that higher doses may lead to adverse effects on cardiac markers. Therefore, it is recommended to use this herbal mixture within a safe and well-defined therapeutic range, and individuals should consult with healthcare professionals for guidance on appropriate dosages. Also, the potential benefits of combining Stone Herbal Mixture with established medications should be considered, particularly those with cardioprotective properties like aspirin. The ability of aspirin to mitigate the adverse effects of the herbal mixture on cardiac markers underscores the importance of combining herbal remedies with evidence-based treatments for cardiovascular health. Additional studies are needed to explore the safety and efficacy of Stone Herbal Mixture in more extensive and diverse populations. This can help establish clearer guidelines for its use and better understand its potential benefits and risks. Regulatory authorities should consider monitoring herbal products like Stone Herbal Mixture to ensure their safety and efficacy. Establishing quality control standards, labeling requirements, and dosage guidelines can help protect consumers. Both healthcare professionals and the public should be educated about the potential risks and benefits of herbal remedies like Stone Herbal Mixture. This includes information on appropriate dosages, potential interactions with other medications, and the importance of seeking professional advice before using herbal remedies for specific health concerns. In addition, manufacturers and distributors of herbal products should be transparent about the composition and quality of their products. Providing clear information on the ingredients and potential side effects can help consumers make informed decisions.

Conflict of Interest
The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions
The entire study procedure was conducted with the involvement of all writers.

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