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The data analysis was conducted using IBM SPSS Statistics 26 software (SPSS Inc.). We rigorously assessed the assumption of homogeneity of variance (homoscedasticity) as part of our analysis. To determine statistical differences between the treatment and negative control groups, we employed a one-way analysis of variance (ANOVA), followed by post hoc Tukey's test for multiple comparisons. A significance threshold of p < 0.05 was applied to define statistical significance. For your reference, detailed statistical results, including the ANOVA F-value, degrees of freedom, and corresponding p-values, are presented in Table S1-S6.

Table S1 One-way ANOVA result for heart rate at 24 hpf of the ZAS-treated groups and the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	319.611	5	63.922	0.883	0.521
Within Groups	868.667	12	72.389		
Total	1188.278	17			

Table S2 One-way ANOVA result for heart rate at 48 hpf of the ZAS-treated groups and the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	471.778	5	94.356	0.700	0.634
Within Groups	1617.333	12	134.778		
Total	2089.111	17			

Table S3 One-way ANOVA result for heart rate at 24 hpf of the CMS-treated groups and the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	279.111	5	55.822	0.483	0.782
Within Groups	1386.000	12	115.500		
Total	1665.111	17			

Table S4 One-way ANOVA result for heart rate at 48 hpf of the CMS-treated groups and the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	5550.944	5	1110.189	28.920	0.000
Within Groups	460.667	12	38.389		
Total	6011.611	17			

Table S5 One-way ANOVA result for heart rate at 24 hpf of the ZASCMS50-treated groups and the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	122.278	5	24.456	0.796	0.573
Within Groups	368.667	12	30.722		
Total	490.944	17			

Table S6 One-way ANOVA result for heart rate at 48 hpf of the ZASCMS50-treated groups and with the negative control.

Variance	Sum of Squares	Degree of freedom	Mean Square	F	p value
Between Groups	287.111	5	57.422	0.361	0.865
Within Groups	1906.667	12	158.889		
Total	2193.778	17			