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Research Article

Oxidative Stress Induced by Viruddha Kshira Samyogas – Superoxide Dismutase and Glutathione Assay in Zebrafish Liver Tissue Homogenate

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ABSTRACT

Introduction: In *Ayurveda*, the concept of *viruddha ahara* (incompatible food combinations) is a well-established principle that emphasizes the adverse effects of certain dietary pairings on health. One of the most significant concepts in this regard is *kshira viruddha*, the incompatibility of milk with other substances such as *lavana* (salt), *kadali* (banana), *maasha* (black gram), *matsya* (fish), and *dadima* (pomegranate). This study explores the oxidative stress induced by these combinations in zebrafish, to better understand the biochemical consequences of *viruddha ahara*.

Materials and Methods: The zebrafish were divided into seven groups: Group A (kshira with lavana), Group B (kshira with kadali), Group C (kshira with maasha), Group D (kshira with matsya), Group E (kshira with dadima), Group F (kshira alone), and Group G (control group, receiving standard fish feed). The test diets were prepared by homogenizing equal proportions of kshira with the respective incompatible substances and administered to zebrafish in their respective tanks. Superoxide dismutase (SOD) and Glutathione (GSH) levels were measured using liver tissue homogenates.

Results and discussion: The control group (Group G) exhibited optimal oxidative balance. Among the experimental groups, Group D (kshira with matsya) exhibited the lowest SOD activity (31.3 U/mg) and significantly reduced GSH levels (49.5%), indicating severe oxidative stress, followed by Group A (kshira with lavana). Other groups of Kshira viruddha combinations exhibited moderate oxidative stress. The results suggest a varying degree of oxidative stress induced by different kshira combinations.

Discussion and conclusion: The study highlights the physiological disruptions caused by *viruddha kshira* combinations, which are consistent with *Ayurvedic* principles warning against incompatible food pairings. The depletion of GSH and reduced SOD activity in the experimental groups reflect the induction of oxidative stress. These findings provide biochemical evidence for the adverse effects of *viruddha ahara*, aligning with traditional *Ayurvedic* dietary principles and emphasizing the importance of food compatibility in maintaining health.

Keywords: Viruddhahara, Viruddha Kshira Samyogas, Superoxide dismutase Assay, Glutathione Assay, Oxidative Stress

INTRODUCTION

In *Ayurveda*, *Viruddhahara* (incompatible food combinations) is a well-documented concept emphasizing the adverse effects of certain dietary pairings on health. These combinations disrupt normal physiological processes, leading to metabolic imbalances and the production of toxic byproducts. Classical Ayurvedic texts such as *Charaka Samhita* and *Sushruta Samhita* describe numerous *Viruddhahara* combinations that can impair digestion, disrupt the balance of *doshas*, and cause diseases ranging from mild gastrointestinal discomfort to severe systemic disorders.[1,2] Among these, *kshira viruddha* (incompatible milk combinations) is particularly significant due to the unique biochemical and metabolic interactions of milk with other substances like *lavana* (common salt), *kadali* (banana), *maasha* (black gram), *matsya* (fish) and *dadima* (pomegranate).

One of the primary mechanisms underlying the health impacts of *Viruddhahara* is the induction of oxidative stress. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses.[3] ROS, including superoxide anions and hydroxyl radicals, are natural byproducts of cellular metabolism but can become harmful when excessively generated. This imbalance disrupts redox signaling and leads to oxidative damage of cellular components such as lipids, proteins, and DNA, contributing to aging, chronic diseases, and inflammatory conditions. [4,5]

The role of dietary choices in modulating oxidative stress is critical. Certain foods and their combinations can either enhance the body's antioxidant defenses or exacerbate ROS production. [6,7] For instance, diets rich in antioxidants such as glutathione and enzymatic protectors like superoxide dismutase (SOD) help maintain redox balance,

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whereas high-calorie diets or incompatible combinations, such as those described in *Viruddhahara*, may elevate oxidative stress and inflammation. Research indicates that high-calorie and poorly balanced diets are linked to the development of chronic diseases, including obesity, neurodegenerative disorders, and cardiovascular conditions, by promoting ROS production and impairing antioxidant systems. [8]

In this context, the study of *viruddha kshira* combinations and their impact on oxidative stress is essential to understand their biochemical implications. Zebrafish (*Danio rerio*) have emerged as a valuable model for studying oxidative stress due to their well-conserved antioxidant systems, transparent embryos, and the ease of observing real-time physiological changes. This study aims to explore the oxidative stress induced by various viruddha kshira combinations in zebrafish. The combinations of kshira with lavana, kadali, maasha, matsya, and dadima were analyzed for their impact on glutathione levels and SOD activity. Understanding the molecular mechanisms of *viruddhahara* provides an opportunity to bridge traditional wisdom with modern biomedical insights for improved dietary guidelines and health outcomes.

MATERIALS AND METHODS

Zebrafish Husbandry: Twelve-week-old zebrafish (Danio rerio) were acquired and acclimated to laboratory conditions. The fish were housed in standard laboratory tanks with the temperature maintained at 28°C and pH between 7.0-7.5. Appropriate filtration systems were installed to ensure optimal water quality. The zebrafish were maintained under a 14:10 light-dark cycle for two weeks before experimentation.

Experimental Design: The zebrafish were divided into groups based on the type of diet administered for SOD and glutathione assays. The groups were as follows: Group A (kshira with lavana), Group B (kshira with kadali), Group C (kshira with maasha), and Group D (kshira with matsya), Group E (kshira with dadima), Group F was fed kshira alone, while Group G served as the control, receiving standard fish feed. Zebrafish were randomly assigned to experimental tanks with a male-to-female ratio of 2:1. The test diets were prepared by homogenizing equal proportions of kshira with the respective incompatible substances. These mixtures were administered to the zebrafishes in the respective tanks.

SOD Assay: Superoxide dismutase (SOD) activity was measured using liver tissue homogenates from the zebrafish fed with different combinations. The homogenate was diluted with 0.5 ml of water, followed by the addition of 250 µl of ice-cold ethanol and 150 µl of chloroform. The mixture was shaken for 2 minutes and centrifuged at 2000 rpm. From the supernatant, 500 µl was taken and mixed with 100 µl of 75 mM Tris HCl buffer (pH 8.2), 50 µl of 25 mM EDTA, and 50 µl of 1.5 mM pyrogallol. The change in absorbance per minute was recorded at 420 nm using a double-beam UV-Vis spectrophotometer. SOD activity was expressed as units per milligram (u/mg) of protein, with one unit of SOD activity defined based on the rate of inhibition of pyrogallol auto-oxidation.

Glutathione Assay: Glutathione (GSH) levels were measured as an indicator of antioxidant capacity. For the assay, 250 µl of liver tissue homogenate was mixed with 1 ml of 5% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 5 minutes at room temperature. The supernatant (500 µl) was then combined with 500 µl of 1× phosphate-buffered saline (PBS, pH 8) and 500 µl of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), prepared at a concentration of 0.1 mM. The final volume was adjusted to 10 ml with methanol (3.5-6.5 ml). The absorbance of the yellow-colored complex was determined at 412 nm using a spectrophotometer. Glutathione activity was expressed as nmol/mg protein.

RESULTS

The control group (Group G) exhibited optimal oxidative balance, with the highest *glutathione* (GSH) levels (97.2%) and *SOD* activity (87 U/mg protein), indicating strong antioxidant defense mechanisms. Among the experimental groups, *Group D (kshira with matsya)* induced the most severe oxidative stress, with the lowest *SOD* activity (31.3 U/mg) and significantly reduced *GSH* levels (49.5%), suggesting substantial oxidative damage. Similarly, *Group A (kshira with lavana)* also caused notable oxidative imbalance, with *SOD* levels at 66.1 U/mg and the lowest *GSH* levels (44.7%), reinforcing the adverse impact of combining *kshira* with *lavana* or *matsya*. [Table No.1]

Table No. 1: SOD and GSH Assay for Incompatible Food combination in Zebra Fish

Groups	SOD	GSH
Group A (*Kshira* with *Lavana*)	66.1	44.7
Group B (*Kshira* with *Kadali*)	53	45
Group C (*Kshira* with *Maasha*)	58.2	67.1
Group D (*Kshira* with *Matsya*)	31.3	49.5
Group E (*Kshira* with *Dadima*)	54.8	46.7
Group F (*Kshira* Alone)	48.3	57
Group G (Control Group)	87	97.2

In comparison, *Group B (kshira with kadali)* and *Group E (kshira with dadima)* exhibited moderate oxidative stress responses. *GSH* levels were 45.0% and 46.7%, respectively, while *SOD* activity was 53.0 U/mg for *Group B* and 54.8 U/mg for *Group E*. Although the oxidative disruption was less severe than in *Groups A and D*, these combinations still led to noticeable oxidative stress and impaired redox balance. *Group C (kshira with maasha)* demonstrated relatively better antioxidant defense, with the highest *GSH* levels (67.1%) and *SOD* activity at 58.2 U/mg among the experimental groups, indicating a less pronounced impact on oxidative balance.

The severity of oxidative stress varied across the different combinations, with Group D (*kshira-matsya)* and Group A (*kshira-lavana*) causing the most pronounced disruption in antioxidant defense mechanisms, while Group B (*kshira-kadali)*, Group E (*kshira-dadima)*, and Group C (*kshira-*

maasha) induced moderate but significant oxidative effects

DISCUSSION

The study highlights the physiological disruptions caused by viruddha *Kshira* combinations, aligning with Ayurvedic principles that caution against such dietary practices. The observed depletion of glutathione and reduced SOD activity reflects oxidative stress, with the severity varying across different combinations. *Kshira* with *lavana* induced the most pronounced oxidative stress, possibly due to osmotic imbalances and metabolic disruptions caused by high salt content. Similarly, the combination of *Kshira* with *matsya* likely generated synergistic ROS due to interactions between milk and fish proteins. In comparison, *kshira* with *kadali* and *kshira* with *maasha* caused moderate oxidative stress, possibly influenced by their fiber content and antinutritional factors like tannins and phytic acids.

This study underscores the relevance of traditional dietary guidelines in preventing metabolic and oxidative disorders. By using zebrafish as a model, it bridges *Ayurveda*'s conceptual framework with contemporary research, offering a deeper understanding of the biochemical and molecular mechanisms underlying the adverse effects of *viruddhahara.* Future research should focus on elucidating these mechanisms further and exploring potential therapeutic interventions to mitigate the adverse effects of dietary incompatibilities.

CONCLUSION

The principles of *Ayurveda*, particularly the concept of *viruddha ahara*, emphasize the importance of food compatibility in maintaining overall health and preventing oxidative stress-related disorders. Avoiding incompatible food combinations and practicing healthy dietary habits can help preserve *doshic* balance and support optimal physiological functions. Adhering to these traditional guidelines may play a crucial role in reducing metabolic imbalances and promoting long-term well-being.

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