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Comparative evaluation of salivary antioxidant levels in yoga practitioners and non-practitioners: A biochemical study

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Abstract:
Oxidative stress, resulting from an imbalance between reactive oxygen species and antioxidant defence mechanisms, contributes to the pathogenesis of several systemic and oral diseases, while yoga has emerged as a promising non-pharmacological intervention to improve oxidative balance. Therefore, it is of interest to evaluate the salivary antioxidant levels in 60 healthy adults (20–40 years), including 30 regular yoga practitioners and 30 age-matched non-practitioners, using unstimulated saliva samples analysed for Total Antioxidant Capacity (TAC), Uric Acid, Superoxide Dismutase (SOD) and Catalase through standard spectrophotometric assays. Statistical analysis using an independent sample t-test showed significantly higher levels of all antioxidant parameters among yoga practitioners compared with non-practitioners ($p < 0.001$). Thus, data shows that regular yoga practice is associated with enhanced salivary antioxidant activity and reduced oxidative stress.

Keywords: Yoga, oxidative stress, saliva, antioxidants, total antioxidant capacity (TAC), superoxide dismutase, catalase, oral health

Background:
Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's antioxidant defence mechanisms, resulting in disruption of redox homeostasis and oxidative damage to cellular biomolecules including lipids, proteins, carbohydrates and nucleic acids. Excessive oxidative stress contributes to tissue injury, inflammation and cellular dysfunction and has been implicated in the pathogenesis of numerous chronic diseases **[1, 2]**. Growing evidence indicates that oxidative stress plays a significant role in the development and progression of cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, autoimmune diseases, metabolic syndrome and various malignancies. Within the oral cavity, oxidative stress contributes to the pathogenesis of periodontal disease, oral potentially malignant disorders, oral lichen planus, recurrent aphthous stomatitis and oral squamous cell carcinoma through enhanced inflammatory responses and oxidative tissue damage **[2, 3]**. The antioxidant defence system comprises both enzymatic antioxidants, including superoxide dismutase (SOD), catalase and glutathione peroxidase and non-enzymatic antioxidants such as uric acid, glutathione, albumin, vitamins C and E, bilirubin and melatonin. These antioxidant systems function synergistically to neutralise free radicals, maintain redox balance and minimise oxidative damage under physiological conditions **[2]**. Saliva has emerged as a valuable diagnostic biofluid because of its non-invasive collection, ease of handling and ability to reflect both oral and systemic physiological changes. Salivary biomarkers have gained increasing importance for evaluating oxidative stress, inflammation, metabolic disorders and oral diseases. Saliva contains numerous antioxidant components that provide the first line of defence against oxidative injury within the oral cavity and contribute to maintenance of oral homeostasis **[2, 4]**. Total Antioxidant Capacity (TAC) represents the cumulative activity of enzymatic and non-enzymatic antioxidants present in saliva and is considered an overall indicator of antioxidant status. Uric acid accounts for the major proportion of salivary antioxidant activity, whereas SOD catalyses the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified into water and oxygen by catalase. Alterations in these biomarkers reflect changes in oxidative stress and antioxidant defence mechanisms **[2]**. Yoga is a comprehensive mind-body practice that integrates physical postures (asanas), breathing techniques (pranayama), meditation and relaxation practices. Increasing scientific evidence suggests that regular yoga practice improves cardiovascular, metabolic, immune and psychological health while reducing stress and systemic inflammation. These beneficial effects are mediated through modulation of autonomic nervous system activity, attenuation of hypothalamic-pituitary-adrenal axis activation, reduction in cortisol secretion, improvement in mitochondrial efficiency and enhancement of endogenous antioxidant defence mechanisms **[3, 5]**. Recent studies have showed that yogic breathing exercises and regular yoga practice are associated with higher salivary total antioxidant capacity, reduced oxidative stress and improved periodontal health. Although several investigations have evaluated serum oxidative stress markers following yoga interventions, evidence regarding salivary antioxidant biomarkers remains comparatively limited. Considering the increasing importance of salivary diagnostics and the widespread adoption of yoga as a preventive healthcare intervention, further research evaluating salivary antioxidant profiles among yoga practitioners is warranted **[3, 5]**. Therefore, it is of interest to evaluate and compare the salivary antioxidant levels among regular yoga practitioners and non-practitioners and to explore the potential association between regular yoga practice and oxidative stress

Materials and Methods:
Study design:

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Bioinformation 22(8): 5431-5436 (2026)

The present investigation was designed as a cross-sectional comparative biochemical study conducted among healthy adult participants. The study was carried out at Seema Dental College and Hospital, Rishikesh, Uttarakhand, India, over a period of six months following approval from the Institutional Ethics Committee.

Ethical considerations:
The study protocol adhered to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment. Confidentiality and anonymity were maintained throughout the study.

Study population:
A total of 60 healthy individuals aged between 20 and 40 years participated in the study. Participants were divided equally into two groups.

[1] **Group A** – Yoga Practitioners (n = 30): Participants practicing yoga regularly for at least one year, with a minimum frequency of five days per week and a session duration of 45–60 minutes.
[2] **Group B –** Non-Practitioners (n = 30): Healthy individuals with no prior history of yoga, meditation, pranayama, or related mind-body practices.

Inclusion criteria:
[1] Healthy individuals aged 20–40 years
[2] Willingness to participate
[3] Ability to provide informed consent
[4] Absence of systemic illness

Exclusion criteria:
[1] Tobacco use in any form
[2] Alcohol consumption
[3] Active periodontal disease
[4] Oral infections or inflammatory lesions
[5] Antioxidant supplementation
[6] Pregnancy and lactation
[7] Chronic systemic disorders

Study flowchart:
Standardized unstimulated saliva collection using the passive drooling technique:

Yoga practice and antioxidant enhancement:
Participants Assessed for Eligibility (n=75)
↓
Excluded (n=15)
↓
Eligible Participants (n=60)
↓
Yoga Practitioners (n=30) ↔ Non-Practitioners (n=30)
↓
Saliva Collection
↓
Biochemical Analysis
↓
Statistical Evaluation

Saliva collection procedure:
Unstimulated whole saliva was collected using the passive drooling method. Participants were instructed to refrain from eating, drinking, chewing gum, smoking and oral hygiene procedures for at least two hours before sample collection. Samples were collected between 9:00 AM and 11:00 AM to minimize circadian variations in salivary composition. Approximately 5 mL of saliva was collected from each participant in sterile polypropylene tubes and immediately stored at −20°C until biochemical analysis. The antioxidant biomarkers, their biological roles and analytical methods are summarized in **Table 1**.

Table 1: Salivary antioxidant biomarkers assessed

Total Antioxidant Capacity (TAC)	Overall antioxidant defense	FRAP Method
Uric Acid	Major non-enzymatic antioxidant	Uricase–Peroxidase Method
Superoxide Dismutase (SOD)	Scavenging of superoxide radicals	NBT Reduction Method
Catalase	Detoxification of hydrogen peroxide	H_2O_2 Decomposition Method

Table 2: Demographic characteristics of study participants

Variable	Yoga Practitioners (n=30)	Non-Practitioners (n=30)	p-value
Age (Years)	29.4 ± 5.2	28.8 ± 4.9	0.63
Male Participants	16 (53.3%)	15 (50.0%)	0.79
Female Participants	14 (46.7%)	15 (50.0%)	0.79
BMI (kg/m²)	23.1 ± 2.8	23.8 ± 3.1	0.37
Duration of Yoga Practice (Years)	3.8 ± 1.7	NA	–

No statistically significant differences were observed between the groups for baseline demographic variables ($p > 0.05$).

Table 3: Comparison of salivary antioxidant parameters between study groups

Parameter	Yoga Group (Mean ± SD)	Non-Yoga Group (Mean ± SD)	Mean Difference	p-value
Total Antioxidant Capacity (μmol/L)	950 ± 120	710 ± 115	+240	<0.001
Uric Acid (mg/dL)	4.2 ± 0.6	3.1 ± 0.5	+1.1	<0.001
Superoxide Dismutase (U/mL)	6.8 ± 0.9	4.9 ± 0.8	+1.9	<0.001
Catalase (U/mL)	13.5 ± 1.8	10.1 ± 1.6	+3.4	<0.001

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Bioinformation 22(8): 5431-5436 (2026)

Table 4: Percentage Increase in Salivary Antioxidant biomarkers

Biomarker	Percentage Increase (%)
Total Antioxidant Capacity	33.8
Uric Acid	35.5
Superoxide Dismutase	38.8
Catalase	33.7

Table 5: Biological relevance of antioxidant parameters

Biomarker	Biological Function	Clinical Relevance
TAC	Overall antioxidant defense	Indicates cumulative antioxidant protection
Uric Acid	Free radical scavenging	Major salivary antioxidant component
SOD	Converts superoxide radicals into hydrogen peroxide	First line of enzymatic antioxidant defense
Catalase	Converts hydrogen peroxide into water and oxygen	Prevents oxidative cellular injury

Table 6: Proposed mechanisms underlying enhanced antioxidant levels

Yoga Component	Physiological Effect	Antioxidant Outcome
Asanas	Improved circulation	Reduced free radical generation
Pranayama	Enhanced oxygen utilization	Improved mitochondrial efficiency
Meditation	Reduced stress hormone secretion	Lower oxidative stress burden
Relaxation Techniques	Increased parasympathetic activity	Enhanced antioxidant response

Statistical analysis:

Data were entered into Microsoft Excel and analyzed using SPSS software version 25.0. Descriptive statistics including mean and standard deviation were calculated for all variables. Intergroup comparisons were performed using an independent sample t-test. Statistical significance was considered at $p < 0.05$.

Results:

A total of 60 participants were included in the final analysis, comprising 30 regular yoga practitioners (Group A) and 30 non-practitioners (Group B). All participants successfully completed saliva collection and biochemical assessment procedures. No samples were excluded from analysis. The demographic profile of the study population is presented in **Table 2**. Both groups were comparable with respect to age, gender distribution and body mass index (BMI), indicating adequate matching and minimizing the influence of confounding variables. Yoga practitioners demonstrated significantly higher levels of all evaluated salivary antioxidant biomarkers compared with non-practitioners, with all between-group differences reaching statistical significance ($p < 0.001$) **(Table 3)**. Total Antioxidant Capacity showed the largest absolute difference, whereas Superoxide Dismutase showed the greatest relative difference. The between-group comparison of salivary antioxidant biomarkers is illustrated in **Figure 1**. To quantify the relative between-group differences, percentage increases in antioxidant biomarkers among yoga practitioners were calculated **(Table 4)**. Superoxide Dismutase showed the greatest relative increase (38.8%), followed by uric acid (35.5%), Total Antioxidant Capacity (33.8%) and catalase (33.7%). These differences indicate a higher salivary antioxidant profile among yoga practitioners, as illustrated in **Figure 2**. The biological relevance of the measured antioxidant parameters and the proposed mechanisms underlying the enhanced antioxidant profile are summarized in **Tables 5 and 6**, respectively. The proportional contribution of major salivary antioxidant components is illustrated in **Figure 3**.

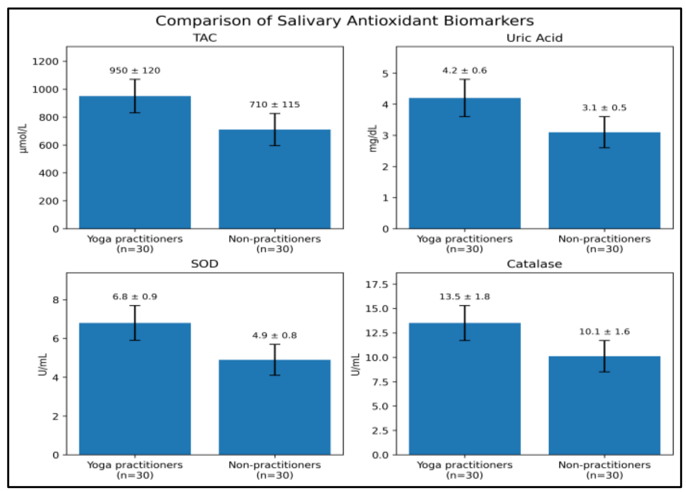

Figure 1: Comparison of salivary antioxidant biomarkers between yoga practitioners and non-practitioners. Bars represent group means and error bars indicate ± standard deviation (SD); each biomarker is displayed on its own scale to preserve readability.

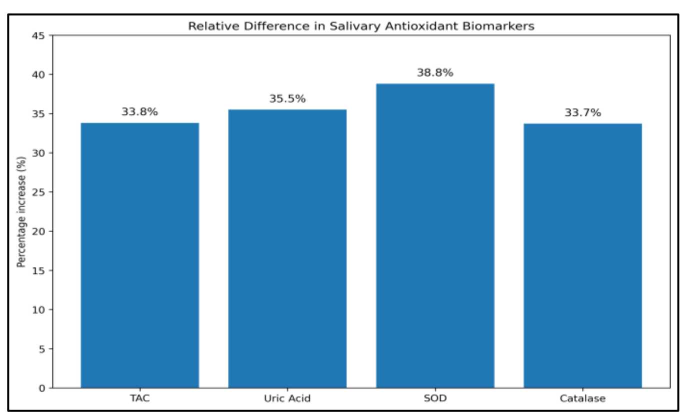

Figure 2: Relative percentage increase in salivary antioxidant biomarkers among yoga practitioners compared with non-practitioners. Percentage increase was calculated as [(Yoga group mean − Non-yoga group mean) / Non-yoga group mean] × 100. SOD showed the greatest relative increase (38.8%), followed by uric acid (35.5%), TAC (33.8%) and catalase (33.7%).

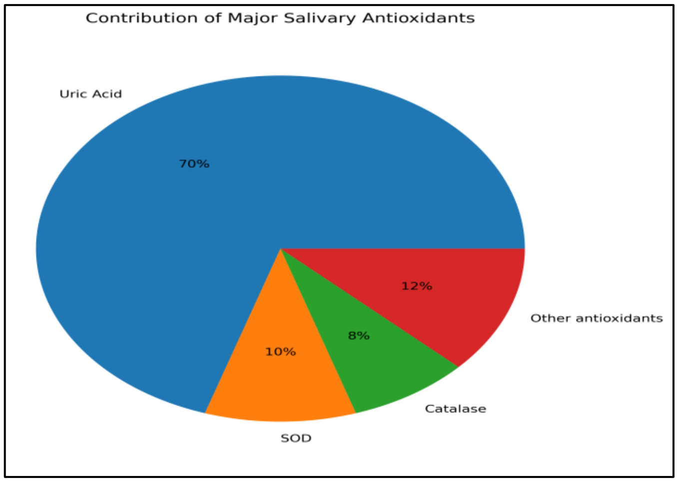

Figure 3: Proportional contribution of major salivary antioxidant components, showing uric acid as the predominant component (70%), followed by other antioxidants (12%), SOD (10%) and catalase (8%).

Discussion:

The present study was undertaken to comparatively evaluate salivary antioxidant levels among regular yoga practitioners and non-practitioners. Oxidative stress has emerged as a critical factor in the pathogenesis of numerous oral and systemic diseases and interventions capable of enhancing endogenous antioxidant defenses have gained considerable scientific interest **[2, 6]**. The findings of the present investigation showed significantly higher levels of Total Antioxidant Capacity (TAC), Uric Acid, Superoxide Dismutase (SOD) and Catalase among yoga practitioners compared with non-practitioners. These results suggest that regular yoga practice may contribute to improved antioxidant defense mechanisms and reduced oxidative stress **[1].** Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant systems. Although ROS are generated during normal metabolic processes, excessive accumulation may cause oxidative damage to lipids, proteins, DNA and cellular membranes. Persistent oxidative stress has been implicated in the development of cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, inflammatory conditions and various oral diseases **[7, 8].** Consequently, strategies that reduce ROS generation or enhance antioxidant activity may play a significant role in disease prevention and health promotion **[9]**. Total Antioxidant Capacity (TAC) reflects the collective effect of enzymatic and non-enzymatic antioxidants in bodily fluids. In this study, higher TAC values were recorded among yoga practitioners than among non-practitioners. Higher TAC indicates a stronger capacity to neutralize free radicals and maintain redox balance **[10]**. The higher TAC observed among yoga practitioners may be related to reduce oxidative stress associated with regular yoga practice, consistent with previous reports of improved antioxidant status following yoga-based interventions **[1, 10]**. Uric acid is a major non-enzymatic antioxidant in saliva and contributes substantially to salivary antioxidant capacity. The significantly higher uric acid concentration among yoga practitioners may indicate enhanced antioxidant protection. Uric acid acts as a scavenger of reactive species, including hydroxyl radicals, singlet oxygen and peroxynitrite, thereby helping limit oxidative injury **[2]**. Similar observations have been reported in studies examining stress reduction and antioxidant status **[11]**. Superoxide dismutase (SOD) is a primary enzymatic defense against oxidative stress and catalyzes the dismutation of superoxide radicals to hydrogen peroxide and oxygen. In the present study, SOD activity was significantly higher among yoga practitioners and showed the greatest relative increase among the biomarkers evaluated **[12, 13]**. Higher SOD activity suggests enhanced enzymatic protection against oxidative injury and may reflect physiological adaptation to regular yoga practice. Catalase is an important antioxidant enzyme that converts hydrogen peroxide into water and oxygen, thereby limiting accumulation of this potentially harmful reactive oxygen species **[15]**. The higher catalase activity observed among yoga practitioners indicates greater capacity to detoxify hydrogen peroxide and may contribute to protection from oxidative cellular injury **[16]**. The concurrent elevation of SOD and catalase supports coordinated enhancement of enzymatic antioxidant defense in the yoga group. Several biological mechanisms may account for the higher antioxidant capacity observed among yoga practitioners. These include modulation of autonomic balance, attenuation of hypothalamic-pituitary-adrenal axis activity, improved mitochondrial efficiency and reduction of systemic inflammation. Yoga may reduce sympathetic tone and enhance parasympathetic activity. Because excessive sympathetic activation is associated with oxidative stress and inflammation, improved autonomic balance may reduce reactive oxygen species production and support endogenous antioxidant defenses **[14, 17 and 18]**. Stress activates the hypothalamic-pituitary-adrenal axis and increases cortisol secretion, which has been linked to oxidative stress and immune dysfunction. Regular yoga practice, including pranayama and meditation, may attenuate HPA-axis activation, reduce circulating cortisol and improve physiological stress responses, thereby supporting antioxidant defense **[14, 19 and 20].** Mitochondria are a major cellular source of reactive oxygen species. Regular yoga practice may improve mitochondrial function, oxygen utilization and bioenergetic efficiency, thereby reducing mitochondrial ROS generation and promoting redox homeostasis **[17, 21]**. Inflammation and oxidative stress are closely interrelated. Yoga has been associated with reductions in pro-inflammatory mediators, and improved antioxidant defense may help limit oxidative tissue injury and support host responses relevant to oral health **[22]**. A systematic review by Syarif *et al*. reported improvement in antioxidant capacity and reduction in oxidative stress with regular yoga practice **[23]**, while an umbrella review by Wang *et al.* described beneficial effects of yoga through oxidative, inflammatory, autonomic and neuroendocrine pathways **[24]**. Salivary antioxidant biomarkers may therefore be useful for evaluating biological responses to lifestyle interventions **[25, 26]**.

Conclusion:

Yoga practitioners had significantly higher salivary antioxidant levels than non-practitioners. Regular yoga practice was associated with stronger antioxidant defence and lower oxidative stress. Yoga may be a useful low-cost adjunct for preventive oral and systemic health.

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