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Effect of Amla (*Emblica officinalis*) Juice Supplementation on Serum Antioxidant Status in Healthy Volunteers: A Single-Group Pre–Post Study

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ABSTRACT:

Background: Oxidative stress, an imbalance between reactive oxygen species and endogenous antioxidant defences, is implicated in the pathogenesis of cardiovascular, metabolic, neurodegenerative and other chronic non-communicable diseases. Amla (*Emblica officinalis*; syn. *Phyllanthus emblica*), a fruit deeply embedded in the Ayurvedic and Siddha traditions of South India, is exceptionally rich in vitamin C and hydrolysable-tannin polyphenols and is a plausible low-cost dietary antioxidant. Direct human data on its effect on serum antioxidant status remain limited.

Objective: To estimate the change in serum enzymatic and non-enzymatic antioxidant markers and a lipid-peroxidation marker after eight weeks of amla juice supplementation in healthy adults.

Methods: In this single-group pre–post interventional study, 50 healthy adult volunteers (31 male, 19 female; mean age 33.4 ± 9.9 years) consumed 30 mL/day of fresh amla juice for 8 weeks. Serum total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase, reduced glutathione (GSH), malondialdehyde (MDA) and vitamin C were

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measured before and after the intervention by standard methods. Paired *t*-tests, 95% confidence intervals (CI) and Cohen's *dz* were computed.

Results: Adherence averaged 91.2%. All antioxidant defence markers rose significantly: TAC 1.232→1.472 mmol/L (+0.240; 95% CI 0.221–0.259), SOD 7.871→9.524 U/mL (+1.653; 1.528–1.778), catalase 41.610→49.840 kU/L (+8.230; 7.687–8.773), GSH 4.518→5.303 mg/dL (+0.786; 0.719–0.852) and vitamin C 0.711→0.960 mg/dL (+0.249; 0.225–0.273); all $p<0.001$. MDA fell from 4.738 to 3.790 nmol/mL (−0.948; 95% CI −1.037 to −0.859; $p<0.001$), indicating reduced lipid peroxidation. Effect sizes were large (*dz* 2.96–4.31). Only mild, self-limiting adverse effects occurred (12/50, 24%); there were no serious events and no discontinuations.

Conclusion: Eight weeks of amla juice was associated with significantly enhanced enzymatic and non-enzymatic antioxidant defences and reduced lipid peroxidation, consistent with amla's high vitamin C and polyphenol content.

1. INTRODUCTION

Oxidative stress arises when the generation of reactive oxygen and nitrogen species outpaces the capacity of endogenous antioxidant defences to neutralize them. The resulting oxidative modification of lipids, proteins and nucleic acids contributes to endothelial dysfunction, chronic low-grade inflammation, insulin resistance and cellular senescence, and is mechanistically linked to cardiovascular disease, type 2 diabetes mellitus, neurodegeneration and several cancers [1,2]. The human antioxidant defence system is conventionally divided into enzymatic components—superoxide dismutase (SOD), catalase and the glutathione-dependent enzymes—and non-enzymatic components, including reduced glutathione (GSH), vitamin C, vitamin E and dietary polyphenols. Total antioxidant capacity (TAC) provides an integrated measure of these overlapping systems, whereas malondialdehyde (MDA), a terminal product of polyunsaturated-fatty-acid peroxidation, is among the most widely used biomarkers of oxidative membrane damage.

Because endogenous defences can be reinforced by exogenous dietary antioxidants, there is longstanding interest in food-based strategies to improve redox balance. Fruits and vegetables that are simultaneously rich in ascorbate and polyphenols are especially attractive, since they can act through complementary mechanisms: direct radical scavenging, metal chelation, and up-regulation of the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway that governs the transcription of endogenous antioxidant enzymes [3,4]. Among such foods, amla occupies a distinctive position.

Amla (*Emblica officinalis* Gaertn.; synonym *Phyllanthus emblica* L.), known regionally as Indian gooseberry, *nellikai* in Tamil and Kannada and *usiri* in Telugu, is a small, translucent green fruit that has been used for more than two millennia in the Ayurvedic and Siddha systems of medicine that remain widely practised across South India. It is a principal ingredient of the classical rejuvenative formulation *Chyawanprash* and of the *triphala* combination, and is traditionally prescribed as a *rasayana* (rejuvenator) believed to promote longevity and vitality. Modern phytochemical analysis has provided a plausible biochemical basis for these traditional claims: amla is one of the richest natural sources of vitamin C and is dense in hydrolysable tannins and phenolic acids, including gallic acid, ellagic acid, chebulagic acid, chebulinic acid, corilagin and the amla-specific low-molecular-weight tannins emblicanin A and emblicanin B [5-8]. Corilagin in particular has attracted attention as a bioactive tannin with antioxidant, anti-inflammatory and hepatoprotective activities.

A substantial preclinical and clinical literature reports antioxidant, anti-inflammatory, antihyperlipidaemic, antihyperglycaemic, hepatoprotective, antimicrobial and immunomodulatory effects of amla and its extracts [9]. A systematic review and meta-analysis of randomized controlled trials found that *Emblica officinalis* supplementation improved lipid profile, fasting glucose and C-reactive protein, supporting a cardiometabolic benefit that is at least partly attributable to reduced oxidative and inflammatory burden [10]. Integrative reviews position amla, alongside ginger, cinnamon and turmeric, among the dietary botanicals with potential for type 2 diabetes prevention [6], and mechanistic animal work suggests amla extract can ameliorate methylglyoxal-associated leptin resistance and related metabolic derangements [7]. Its polyphenol density has additionally been linked to

benefits for oral health [8], to enhanced antioxidant capacity of milk and blood in supplemented dairy cows [9], and to antimicrobial and anti-inflammatory [11] activities across the *Phyllanthus* genus. Despite this breadth of evidence, direct measurements of the effect of a simple, dietary preparation of amla juice on a panel of serum antioxidant markers in healthy human volunteers remain comparatively sparse, and few reports frame the question within the everyday dietary context of South India, where amla is inexpensive, culturally familiar and seasonally abundant.

The present study was therefore designed to estimate, in healthy South Indian adults, the change in serum antioxidant status after eight weeks of daily amla juice supplementation. The specific objective was to quantify pre-to-post changes in enzymatic antioxidants (SOD, catalase), non-enzymatic antioxidants (TAC, GSH, vitamin C) and the lipid-peroxidation marker MDA, and to describe the tolerability of the preparation. We hypothesized that daily amla juice would be associated with an increase in antioxidant defence markers and a reduction in MDA. Given the single-arm, uncontrolled design, the study was conceived as hypothesis-generating rather than confirmatory.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was a single-group (single-arm) pre–post interventional study conducted in the South India, over the study period [v no 5437]. The design compared each participant's serum antioxidant markers before and after the intervention, so that every volunteer served as his or her own control.

2.2 Participants

Fifty apparently healthy adult volunteers of both sexes, aged 20–55 years, were recruited from staff and students of the institution and the surrounding community. Inclusion criteria were: apparently healthy adults aged 20–55 years, able and willing to consume amla juice daily and to provide informed consent. Exclusion criteria were: pregnancy or lactation; current tobacco or heavy alcohol use; acute illness in the preceding two weeks; any chronic disease or chronic medication known to affect oxidative-stress status (including diabetes mellitus, cardiovascular, hepatic or renal disease); current use of antioxidant vitamin or polyphenol supplements; and known intolerance or allergy to amla. Volunteers were asked to maintain their usual diet and physical-activity patterns and to avoid additional antioxidant supplements during the study.

2.3 Intervention

Each participant consumed 30 mL of fresh amla (*Emblica officinalis*) juice once daily for eight consecutive weeks. Adherence was estimated from participant diaries and periodic telephone or in-person follow-up and expressed as the percentage of prescribed doses consumed. Adverse effects reported spontaneously or on direct questioning at follow-up visits were recorded and graded.

2.4 Blood sampling and biochemical assays

Venous blood was drawn after an overnight fast at baseline (before the first dose) and again at the end of week 8. Samples were centrifuged and serum was separated and stored at −80 °C until batched analysis. The following markers were measured by standard, previously validated methods:

- **Total antioxidant capacity (TAC)** — by a ferric-reducing/phosphomolybdenum-type colorimetric assay, expressed in mmol/L.
- **Superoxide dismutase (SOD)** — by an assay based on inhibition of superoxide-mediated chromogen reduction, expressed in U/mL.
- **Catalase** — by measurement of the rate of hydrogen-peroxide decomposition, expressed in kU/L.
- **Reduced glutathione (GSH)** — by the 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman) method, expressed in mg/dL.
- **Malondialdehyde (MDA)** — by the thiobarbituric-acid–reactive-substances (TBARS) method, expressed in nmol/mL.
- **Vitamin C (ascorbic acid)** — by a 2,4-dinitrophenylhydrazine colorimetric method, expressed in mg/dL.

All assays were performed in duplicate with appropriate internal quality controls, and pre- and post-intervention samples for a given participant were assayed within the same batch to minimize inter-assay variability.

2.5 Statistical analysis

Data were compiled in a spreadsheet and analysed with standard statistical software. Continuous variables are summarized as mean ± standard deviation (SD); categorical variables as counts and percentages. For each marker, the pre-to-post change was assessed with a paired *t*-test (degrees of freedom = 49), and the mean difference is reported with its 95% confidence interval (CI). The standardized effect size for paired data, Cohen's *dz* (mean change divided by the SD of the change scores), was calculated for each marker and interpreted using conventional thresholds (0.2 small, 0.5 medium, 0.8 large). A two-sided *p*-value < 0.05 was considered statistically significant. Because the study was single-arm, no between-group comparisons were possible and all inferences pertain to within-participant change.

3. RESULTS

3.1 Baseline characteristics

Fifty volunteers were enrolled and all completed the eight-week intervention, with no discontinuations. The sample comprised 31 men (62%) and 19 women (38%) with a mean age of 33.4 ± 9.9 years. Mean adherence to the prescribed daily amla juice was high at 91.2%. Baseline characteristics are summarized in **Table 1**.

Table 1. Baseline characteristics of the 50 healthy volunteers.

Characteristic	Value
Sample size (n)	50
Age, years (mean ± SD)	33.4 ± 9.9
Sex — male, n (%)	31 (62.0)
Sex — female, n (%)	19 (38.0)
Amla juice dose	30 mL/day
Intervention duration	8 weeks
Mean adherence (%)	91.2
Completed study, n (%)	50 (100.0)

3.2 Enzymatic antioxidant markers

Both enzymatic antioxidants increased significantly after eight weeks (**Table 2**). Serum SOD rose from 7.871 ± 1.022 to 9.524 ± 0.980 U/mL, a mean increase of 1.653 U/mL (95% CI 1.528–1.778; $t = 26.67$, df = 49; $p < 0.001$), with a very large effect size ($dz = 3.77$). Catalase increased from 41.610 ± 5.496 to 49.840 ± 5.665 kU/L, a mean rise of 8.230 kU/L (95% CI 7.687–8.773; $t = 30.47$, df = 49; $p < 0.001$; $dz = 4.31$)—the largest standardized effect of any marker.

Table 2. Enzymatic antioxidant markers before and after 8 weeks of amla juice (paired analysis, N = 50, df = 49).

Marker (unit)	Pre (mean ± SD)	Post (mean ± SD)	Mean change (95% CI)	*t*	*p*	Cohen's *dz*
SOD (U/mL)	7.871 ± 1.022	9.524 ± 0.980	+1.653 (1.528–1.778)	26.67	<0.001	3.77
Catalase (kU/L)	41.610 ± 5.496	49.840 ± 5.665	+8.230 (7.687–8.773)	30.47	<0.001	4.31

3.3 Non-enzymatic markers and lipid peroxidation

The non-enzymatic antioxidant markers likewise improved, and the lipid-peroxidation marker MDA fell (**Table 3**). TAC increased from 1.232 ± 0.182 to 1.472 ± 0.177 mmol/L (+0.240; 95% CI 0.221–0.259; $t = 25.26$; $p < 0.001$; $dz = 3.57$). GSH rose from 4.518 ± 0.588 to 5.303 ± 0.612 mg/dL (+0.786; 95% CI 0.719–0.852; $t = 23.70$; $p < 0.001$; $dz = 3.35$). Vitamin C

increased from 0.711 ± 0.139 to 0.960 ± 0.143 mg/dL (+0.249; 95% CI 0.225–0.273; $t = 20.96$; $p < 0.001$; $dz = 2.96$). In parallel, serum MDA decreased from 4.738 ± 0.600 to 3.790 ± 0.637 nmol/mL, a mean reduction of 0.948 nmol/mL (95% CI −1.037 to −0.859; $t = -21.30$; $p < 0.001$; $dz = -3.01$), consistent with reduced lipid peroxidation.

Table 3. Non-enzymatic antioxidant markers and lipid peroxidation before and after 8 weeks of amla juice (paired analysis, N = 50, df = 49).

Marker (unit)	Pre (mean ± SD)	Post (mean ± SD)	Mean change (95% CI)	*t*	*p*	Cohen's *dz*
TAC (mmol/L)	1.232 ± 0.182	1.472 ± 0.177	+0.240 (0.221–0.259)	25.26	<0.001	3.57
GSH (mg/dL)	4.518 ± 0.588	5.303 ± 0.612	+0.786 (0.719–0.852)	23.70	<0.001	3.35
Vitamin C (mg/dL)	0.711 ± 0.139	0.960 ± 0.143	+0.249 (0.225–0.273)	20.96	<0.001	2.96
MDA (nmol/mL)	4.738 ± 0.600	3.790 ± 0.637	−0.948 (−1.037 to −0.859)	−21.30	<0.001	−3.01

The relative magnitude of change was substantial for every marker: catalase rose by approximately 19.8%, SOD by 21.0%, TAC by 19.5%, GSH by 17.4% and vitamin C by 35.0%, while MDA fell by approximately 20.0%. The pre-versus-post pattern for the five antioxidant defence markers is displayed in **Figure 1**, which presents the paired means on a logarithmic scale (to accommodate the differing measurement units) alongside the percentage change from baseline. The reduction in lipid peroxidation is shown in **Figure 2**.

Figure 1. Serum antioxidant defence markers before and after 8 weeks of amla juice (30 mL/day), N=50

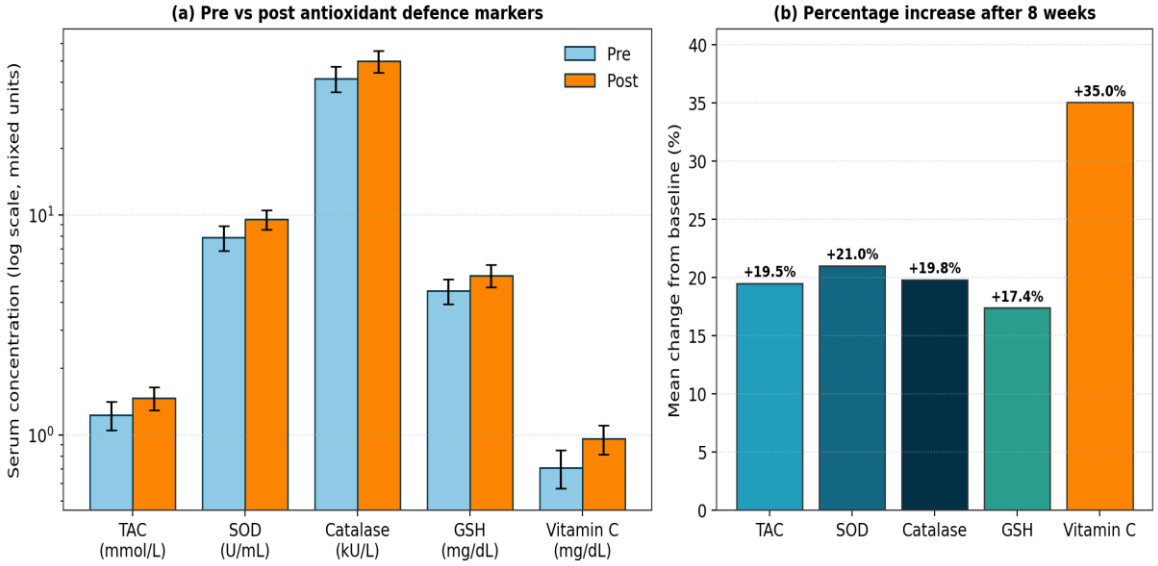

Figure 1. Serum antioxidant defence markers (TAC, SOD, catalase, GSH, vitamin C) before and after 8 weeks of amla juice supplementation (30 mL/day; N = 50). Panel (a) shows paired means with SD error bars on a logarithmic axis to allow markers with different units to be compared; panel (b) shows the mean percentage increase from baseline. All increases were statistically significant ($p < 0.001$).

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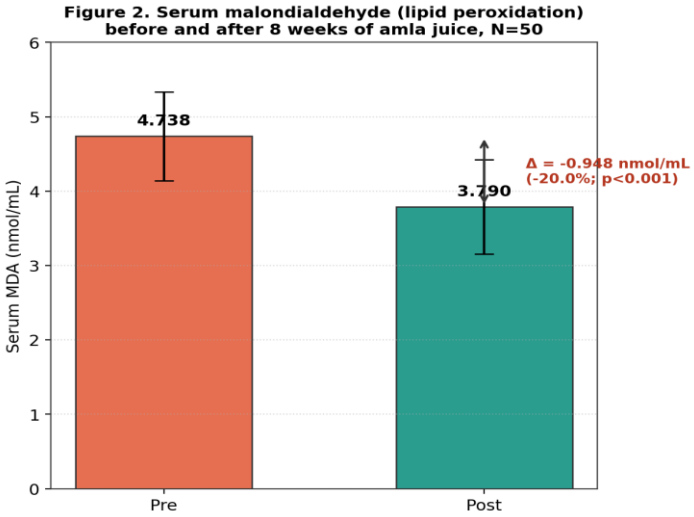

Figure 2. Serum malondialdehyde (MDA), a marker of lipid peroxidation, before and after 8 weeks of amla juice supplementation (30 mL/day; N = 50). MDA fell by 0.948 nmol/mL (−20.0%; $p < 0.001$). Error bars represent SD.

3.3 Adverse effects and tolerability

Amla juice was well tolerated. Mild, self-limiting adverse effects were reported by 12 of 50 participants (24%): mild acidity in 7, transient nausea in 3 and loose stools in 2 (**Table 4**). No serious adverse events occurred, and no participant discontinued the intervention.

Table 4. Adverse effects reported during the 8-week intervention (N = 50).

Adverse effect	n	% of participants
Mild acidity	7	14.0
Transient nausea	3	6.0
Loose stools	2	4.0
Any adverse effect	12	24.0
Serious adverse events	0	0.0
Discontinuations	0	0.0

4. DISCUSSION

In this single-group pre–post study of 50 healthy South Indian adults, eight weeks of daily amla (*Emblica officinalis*) juice supplementation was associated with statistically significant and quantitatively large improvements across the whole panel of serum antioxidant markers examined. Both enzymatic antioxidants (SOD and catalase) and non-enzymatic antioxidants (TAC, GSH and vitamin C) rose, and the lipid-peroxidation marker MDA fell. The standardized effect sizes were uniformly large (*dz* 2.96–4.31), and adherence was high while tolerability was excellent. Taken together, the pattern describes a coordinated shift toward a more reducing serum antioxidant environment after amla supplementation.

4.1 Biochemical plausibility and mechanisms

The observed changes are biologically coherent with the known composition of amla. The fruit is one of the most concentrated natural dietary sources of vitamin C, and the marked rise in serum vitamin C (+35%) is the most direct signal that the intervention delivered bioavailable ascorbate [12,3]. Vitamin C is a potent aqueous-phase radical scavenger and also regenerates other antioxidants, which helps explain the parallel increase in integrated TAC. Beyond ascorbate, amla is exceptionally rich in

hydrolysable tannins and phenolic acids—gallic acid, ellagic acid, chebulagic and chebulinic acids, corilagin and the emblicanins—that contribute both direct radical-scavenging activity and metal-chelating capacity [13]. Corilagin, a bioactive tannin abundant in the *Phyllanthus* genus, has documented antioxidant and hepatoprotective properties and has been highlighted as a molecule of translational interest [14,15].

A widely proposed mechanism is that amla polyphenols activate the Nrf2–antioxidant-response-element signalling axis, up-regulating transcription of endogenous antioxidant and phase-II detoxifying enzymes [2,16]. The concurrent increase in GSH—a central intracellular thiol antioxidant and a substrate for glutathione-dependent enzymes—is consistent with such an adaptive, transcriptionally mediated response. The fall in MDA integrates these effects: with more circulating scavengers and greater enzymatic capacity, the propagation of lipid-peroxidation chain reactions would be expected to diminish, lowering the terminal TBARS signal.

4.2 Relationship to the existing amla literature

The direction of our findings aligns with a broad experimental and clinical literature on amla. A systematic review and meta-analysis of randomized controlled trials reported that *Emblica officinalis* improved lipid profile, fasting glucose and C-reactive protein—cardiometabolic benefits that plausibly share an antioxidant and anti-inflammatory basis with the redox improvements seen here [17]. Comprehensive reviews of the phytochemistry and pharmacology of *Phyllanthus emblica* consistently emphasize its antioxidant activity as a defining property and catalogue the vitamin-C and polyphenol constituents that would drive it [4,18]. Integrative reviews place amla among dietary botanicals with potential for type 2 diabetes prevention [6], and mechanistic animal studies show that amla extract can mitigate methylglyoxal-associated metabolic dysfunction [7], further linking its polyphenols to improved redox and metabolic homeostasis. Evidence from other systems is convergent: polyphenol-rich amla has shown benefits for oral health [8]; fresh amla supplementation enhanced the antioxidant capacity of both milk and blood in dairy cows [19]; and reviews across the *Phyllanthus* genus document antimicrobial [10] and anti-inflammatory [20,21] activities that frequently accompany antioxidant effects. Our human data, though uncontrolled, add a simple, dietary-preparation perspective to this literature by profiling six complementary serum markers simultaneously.

4.3 South Indian dietary and public-health context

These findings are of particular interest in the South Indian context. Amla (*nellikai*/*usiri*) is inexpensive, seasonally abundant and culturally embedded in the Ayurvedic and Siddha traditions that remain in everyday use across the region; it is consumed fresh, pickled, candied, as juice, and within classical formulations such as *Chyawanprash* and *triphala*. A low-cost, familiar, food-based intervention that can plausibly strengthen antioxidant defences is attractive for a population facing a rising burden of oxidative-stress-related non-communicable diseases, including diabetes and cardiovascular disease [22,23].

5. CONCLUSION

In this single-group pre–post study of 50 healthy adults from South India, eight weeks of daily amla (*Emblica officinalis*) juice supplementation was associated with significant, large-magnitude increases in enzymatic (SOD, catalase) and non-enzymatic (TAC, GSH, vitamin C) antioxidant markers and a significant reduction in the lipid-peroxidation marker MDA, with excellent tolerability. These changes are biochemically consistent with amla's exceptionally high Vitamin-C and polyphenol content and with its traditional standing as a *rasayana* in South Indian medicine.

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