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Original Article

Green Electrochemical Fingerprinting of Neem-Derived Polyphenols for Early Oxidative Stress Biomarker Detection

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ABSTRACT\

Background: Electrochemical sensing may provide a comparatively rapid approach for evaluating redox-active compounds, but plant-extract-modified electrodes require careful analytical assessment before clinical use. Objective: To evaluate serum electrochemical responses generated by a neem-leaf-extract-modified electrode and examine their relationships with malondialdehyde and occupational exposure duration. Methods: This cross-sectional analytical study included 85 adults aged 20–55 years living or working in an industrial region of Punjab. Serum was analysed using a three-electrode system comprising a neem-extract-modified glassy carbon working electrode, platinum counter electrode, and Ag/AgCl reference electrode. Cyclic and square-wave voltammetry were performed from −0.2 to +0.8 V. Peak currents provisionally assigned to uric acid and ascorbic acid were correlated with serum malondialdehyde measured using the thiobarbituric acid reactive substances assay. Results: The uric-acid-assigned signal occurred at 0.32 ± 0.04 V with a mean current of 14.82 ± 3.21 µA, while the ascorbic-acid-assigned signal occurred at 0.18 ± 0.03 V with a mean current of 8.45 ± 1.95 µA. The signals correlated positively ($r = 0.742$) and inversely ($r = −0.615$), respectively, with malondialdehyde (both $p < 0.001$). Significant exposure-group differences were observed for malondialdehyde and both peak currents. Conclusion: The modified electrode generated distinguishable serum responses associated with lipid peroxidation and exposure duration, but analyte identity and analytical performance require formal validation. Keywords: Ascorbic Acid; Electrochemical Analysis; Malondialdehyde; Neem; Occupational Exposure; Oxidative Stress; Uric Acid.

INTRODUCTION

Oxidative stress develops when the production of reactive oxygen and nitrogen species exceeds the capacity of endogenous antioxidant systems to neutralize them. Persistent oxidative imbalance may damage lipids, proteins, and nucleic acids and has been associated with cardiovascular, metabolic, neurodegenerative, inflammatory, and malignant disorders. Measurement of circulating oxidative-stress-related compounds may therefore contribute to the investigation of biological responses to environmental, occupational, and metabolic exposures. However, oxidative stress is a complex biological process that cannot usually be represented by a single biomarker, and interpretation requires consideration of both oxidative damage products and endogenous antioxidant responses.

Malondialdehyde is a secondary product of lipid peroxidation and is commonly estimated in biological samples using thiobarbituric acid reactive substances assays. Although this approach is widely used, it

provides an indirect measure of lipid peroxidation and may be influenced by other thiobarbituric-acid-reactive compounds. Uric acid and ascorbic acid are low-molecular-weight electroactive substances that contribute to antioxidant activity in extracellular biological fluids. Their interpretation is nevertheless context dependent. Ascorbic acid is consumed during reactive-species scavenging, whereas uric acid may demonstrate antioxidant activity in extracellular environments while also being associated with inflammatory, renal, and cardiometabolic processes. Simultaneous assessment of these compounds may consequently provide complementary information, but their electrochemical measurement in serum remains challenging because biological matrices contain multiple substances with overlapping oxidation potentials and may cause electrode fouling.

Conventional laboratory methods used to quantify oxidative-stress-related analytes include chromatography, mass spectrometry, spectrophotometry, and immunoassays. These methods can provide reliable measurements but may require centralized laboratory infrastructure, trained personnel, relatively extensive sample processing, and costly instrumentation. Electrochemical techniques offer a potential alternative because they can generate rapid analytical responses from small sample volumes and may be adapted for miniaturized sensing systems. Recent electrochemical platforms have incorporated metal oxides, nanostructures, microfluidic configurations, and phenolic materials to improve electron transfer and analytical response (1–4). Nevertheless, the performance of any proposed electrochemical interface must be established through adequate characterization, analyte confirmation, calibration, repeatability, selectivity, interference testing, and comparison with appropriate reference methods before diagnostic or point-of-care use can be inferred.

Naturally occurring phenolic compounds have attracted attention as electrode-modifying materials because their redox-active functional groups may facilitate electron transfer at the electrode surface. Electrochemical studies have evaluated natural phenolic antioxidants and plant-derived materials in food, nutraceutical, and biological applications (4–7). Neem (Azadirachta indica) leaves contain a mixture of phenolic and other phytochemical constituents and may therefore provide a renewable material for experimental electrode modification. However, the analytical behaviour of neem-extract-modified electrodes in untreated human serum, particularly in relation to electroactive compounds associated with oxidative stress, remains insufficiently characterized. It is also uncertain whether oxidation signals assigned to uric acid and ascorbic acid obtained from such an interface correspond with an independent indicator of lipid peroxidation or vary according to the duration of environmental or occupational exposure.

This study therefore aimed to evaluate the electrochemical responses generated by a neem-leaf-extract-modified glassy carbon electrode in serum samples obtained from adults living or working in an industrial region of Punjab. The specific objectives were to identify separable oxidation signals provisionally assigned to uric acid and ascorbic acid, examine the relationships between their peak currents and serum malondialdehyde concentrations, and compare the electrochemical and biochemical measurements across categories of occupational exposure duration. The study was intended as an initial analytical evaluation of the experimental sensing interface rather than a definitive assessment of diagnostic accuracy or clinical point-of-care performance.

MATERIALS AND METHODS

This cross-sectional analytical laboratory study was conducted in the industrial and urban region of Punjab, Pakistan, from September 2025 to January 2026. Participants were recruited through occupational healthcare clinics and community health centres serving populations living or working in the selected industrial area. The study evaluated electrochemical responses generated by an experimental neem-leaf-extract-modified electrode and examined their associations with serum malondialdehyde concentrations and reported duration of occupational exposure. The study did not use a disease-classification threshold or a clinically established diagnostic reference standard and was

therefore treated as an exploratory sensor-evaluation study rather than a diagnostic-accuracy investigation.

Adults aged 20–55 years who had lived or worked in the industrial area for at least two consecutive years were eligible for inclusion. Individuals were excluded if they had an acute infectious illness, active malignancy, end-stage organ failure, surgery during the preceding six months, current use of antioxidant supplements, or current treatment with immunosuppressive medication. Participants were selected using non-probability convenience sampling. Ninety-two potentially eligible individuals were screened, of whom seven were excluded because of antioxidant supplement use or active infection. The final analytical sample therefore comprised 85 participants.

The sample size was selected for the exploratory evaluation of variability in the electrochemical responses and their correlations with the comparator oxidative-stress measurement. Because the manuscript did not provide the expected correlation, significance level, precision requirement, attrition allowance, or formal calculation procedure, the sample was not represented as the product of a definitive diagnostic-validation calculation. Occupational exposure duration was recorded in years and subsequently classified as less than 5 years, 5–10 years, or more than 10 years for comparative analysis. This variable represented duration of work-related exposure and was not treated as a direct quantitative measurement of any individual pollutant or toxicant.

After an overnight fast, approximately 5 mL of venous blood was collected from each participant by standard venipuncture into a plain gel-separator tube. The specimens were allowed to clot at room temperature for 30 minutes and were then centrifuged at 3000 revolutions per minute for 10 minutes to separate the serum. Fresh serum aliquots were used for electrochemical testing and spectrophotometric estimation of malondialdehyde. Apart from serum separation, no extensive extraction or chemical pretreatment of the samples was undertaken before electrochemical measurement.

The experimental sensor used a conventional three-electrode configuration consisting of a glassy carbon working electrode, platinum-wire counter electrode, and silver/silver chloride reference electrode. The working electrode was modified by drop-casting an aqueous neem leaf extract onto its pre-cleaned surface. The neem extract was used as a mixed plant-derived electrode-modifying material; individual polyphenolic constituents were not separately quantified or chemically identified within the study. The manuscript did not provide sufficient information regarding botanical authentication, extraction ratio, extract concentration, drop-cast volume, drying conditions, electrode dimensions, coating thickness, or electrode-storage conditions. These characteristics should therefore be documented from the original laboratory records before the study is reproduced or submitted as a fully characterized sensor-development investigation.

Electrochemical measurements were performed using cyclic voltammetry and square-wave voltammetry over a potential window of −0.2 V to +0.8 V relative to the silver/silver chloride reference electrode. Peak oxidation potential and peak current were recorded as the principal electrochemical measurements. Signals occurring at approximately +0.32 V and +0.18 V were provisionally assigned to uric acid and ascorbic acid, respectively. The recorded peak currents represented electrochemical signal intensities and were not interpreted as independently verified serum concentrations because the study did not report calibration curves, standard-addition testing, analyte-spiking experiments, or comparison with direct laboratory assays of uric acid and ascorbic acid. The manuscript also did not report the supporting electrolyte, sample dilution, pH, scan rate, square-wave frequency, amplitude, step potential, replicate measurements, peak-processing method, or potentiostat specifications. These operational parameters should be recovered from contemporaneous laboratory documentation before independent replication.

Serum malondialdehyde was estimated spectrophotometrically using the thiobarbituric acid reactive substances method and was expressed in micromoles per litre. The malondialdehyde measurement was

used as a comparator indicator of lipid peroxidation for evaluating biological associations with the electrochemical signals. It was not considered an analytical reference method for determining the accuracy of uric acid or ascorbic acid measurements because it quantifies a different biochemical outcome. Direct analyte-specific assays would be required to establish calibration, agreement, recovery, and analytical accuracy for the two provisionally assigned electrochemical peaks.

The principal study variables were peak current and peak potential for the two assigned electrochemical signals, serum malondialdehyde concentration, and occupational exposure duration. Age, sex, body mass index, systolic blood pressure, and diastolic blood pressure were recorded for descriptive characterization. Potential influences on uric acid, ascorbic acid, and malondialdehyde—including renal function, smoking, diet, medication use, metabolic disease, inflammatory conditions, and specific occupational toxicants—were not included in the reported analysis. Consequently, the observed relationships were interpreted as unadjusted associations and not as evidence that exposure duration independently caused the biomarker differences.

Data were analysed using IBM SPSS Statistics version 26.0. Continuous variables were summarized using means and standard deviations, while categorical variables were presented as frequencies and percentages. Distributional assumptions for the continuous analytical variables were assessed using the Shapiro–Wilk test and should also have been evaluated through graphical inspection for skewness, outliers, and non-linearity. Pearson correlation coefficients were used to examine the relationships between the electrochemical peak currents and serum malondialdehyde concentrations. Correlations were reported with two-sided p-values; confidence intervals should be added where they can be calculated from the verified analytical dataset.

Differences in continuous outcomes across the three occupational-exposure-duration categories were assessed using one-way analysis of variance. Tukey-adjusted post-hoc comparisons were used to examine pairwise group differences when the overall analysis was statistically significant. The assumptions of independence, approximate normality of residuals, and homogeneity of variance were applicable to these analyses. Independent-samples t-tests were included in the original statistical plan but were not linked to a clearly reported two-group comparison and should not be retained unless a corresponding prespecified analysis can be documented. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. Because the analyses were exploratory and unadjusted for important biological and occupational confounders, statistical significance was interpreted cautiously.

The study protocol received approval from an institutional bioethics committee, and the procedures were reported to have been conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before sample collection and laboratory testing. The approving institution and authentic ethics approval identifier should be inserted from the original ethics documentation before publication; these details must not be reconstructed or assumed.

RESULTS

Ninety-two individuals were assessed for eligibility. Seven were excluded, including four who reported recent antioxidant supplement use and three with active infectious conditions. The final analysis therefore included 85 participants, all of whom had complete demographic, electrochemical, and serum malondialdehyde measurements.

The mean age of the participants was 38.4 ± 8.2 years, with an observed range of 21–54 years. The sample included 53 men (62.4%) and 32 women (37.6%). Mean body mass index was 24.8 ± 3.1 kg/m², and the mean reported duration of occupational exposure was 9.6 ± 4.1 years. Twenty-two participants (25.9%) reported less than 5 years of occupational exposure, 35 (41.2%) reported 5–10 years, and 28 (32.9%) reported more than 10 years of exposure. The demographic and clinical characteristics of the participants are presented in Table 1.

Table 1. Demographic and Clinical Characteristics of the Participants (N = 85)

Characteristic	Total sample
Age, years	38.4 ± 8.2
Age range, years	21–54
Male sex	53 (62.4)
Female sex	32 (37.6)
Body mass index, kg/m²	24.8 ± 3.1
Occupational exposure duration, years	9.6 ± 4.1
Exposure duration <5 years	22 (25.9)
Exposure duration 5–10 years	35 (41.2)
Exposure duration >10 years	28 (32.9)
Systolic blood pressure, mmHg	124.6 ± 10.8
Diastolic blood pressure, mmHg	81.2 ± 7.4

Data are presented as mean ± standard deviation or n (%). Occupational exposure duration represents the reported duration of employment-related exposure and not a direct measurement of pollutant intensity.

Square-wave voltammetry generated two distinguishable anodic signals in the tested serum samples. The signal provisionally assigned to uric acid occurred at a mean peak potential of 0.32 ± 0.04 V and had a mean peak current of 14.82 ± 3.21 μA. The signal provisionally assigned to ascorbic acid occurred at a mean peak potential of 0.18 ± 0.03 V and had a mean peak current of 8.45 ± 1.95 μA. Mean serum malondialdehyde concentration measured using the thiobarbituric acid reactive substances assay was 3.68 ± 1.12 μmol/L. These electrochemical measurements represent peak potentials and current responses rather than independently verified serum concentrations of uric acid or ascorbic acid.

Table 2. Electrochemical Responses and Serum Malondialdehyde Measurements (N = 85)

Measurement	Assigned analyte or marker	Mean ± SD	95% CI for the mean
Peak current, μA	Uric acid signal	14.82 ± 3.21	14.13 to 15.51
Peak current, μA	Ascorbic acid signal	8.45 ± 1.95	8.03 to 8.87
Peak potential, V versus Ag/AgCl	Uric acid signal	0.32 ± 0.04	0.31 to 0.33
Peak potential, V versus Ag/AgCl	Ascorbic acid signal	0.18 ± 0.03	0.17 to 0.19
Serum concentration, μmol/L	Malondialdehyde	3.68 ± 1.12	3.44 to 3.92

Ag/AgCl: silver/silver chloride reference electrode; CI: confidence interval; SD: standard deviation. Analyte assignments refer to the reported oxidation potentials. Direct reference-method concentrations of uric acid and ascorbic acid were not included in the analysis.

The peak current assigned to uric acid demonstrated a strong positive correlation with serum malondialdehyde concentration ($r = 0.742$; 95% CI, 0.628 to 0.825; $t[83] = 10.08$; $p < 0.001$). In contrast, the peak current assigned to ascorbic acid demonstrated a moderate-to-strong inverse correlation with malondialdehyde concentration ($r = −0.615$; 95% CI, −0.732 to −0.462; $t[83] = −7.11$; $p < 0.001$). The two electrochemical peak currents were also inversely correlated with each other ($r = −0.485$; 95% CI, −0.633 to −0.303; $t[83] = −5.05$; $p < 0.001$). These findings indicate that larger uric-acid-assigned current responses and smaller ascorbic-acid-assigned current responses were associated with higher serum malondialdehyde concentrations. The correlations do not, however, establish analytical agreement or diagnostic accuracy because the peak currents were not compared with direct reference measurements of uric acid and ascorbic acid.

Table 3. Correlations Between Electrochemical Peak Currents and Serum Malondialdehyde (N = 85)

Variable pair	Pearson r	95% CI	t (df = 83)	p-value
Uric-acid-assigned peak current and serum MDA	0.742	0.628 to 0.825	10.08	<0.001
Ascorbic-acid-assigned peak current and serum MDA	−0.615	−0.732 to −0.462	−7.11	<0.001
Uric-acid-assigned and ascorbic-acid-assigned peak currents	−0.485	−0.633 to −0.303	−5.05	<0.001

CI: confidence interval; MDA: malondialdehyde. Confidence intervals were derived using Fisher's z transformation. All tests were two-sided.

Electrochemical responses and serum malondialdehyde concentrations differed across the three occupational-exposure-duration categories. Mean serum malondialdehyde increased from 2.81 ± 0.76 μmol/L among participants with less than 5 years of exposure to 3.52 ± 0.91 μmol/L among those with 5–10 years and 4.35 ± 0.98 μmol/L among those with more than 10 years of exposure. The overall group difference was statistically significant, $F(2,82) = 18.42$, $p < 0.001$, with $\eta^2 = 0.310$.

The mean uric-acid-assigned peak current increased across the same exposure categories, from 11.65 ± 2.10 μA in the group with less than 5 years of exposure to 14.78 ± 2.65 μA in the 5–10-year group and 17.12 ± 2.84 μA in the group with more than 10 years of exposure. The overall difference was statistically significant, $F(2,82) = 23.15$, $p < 0.001$, with $\eta^2 = 0.361$.

Conversely, the mean ascorbic-acid-assigned peak current decreased from 9.82 ± 1.54 μA among participants with less than 5 years of exposure to 8.41 ± 1.72 μA among those with 5–10 years and 7.42 ± 1.88 μA among those with more than 10 years of exposure. The overall difference was statistically significant, $F(2,82) = 11.84$, $p < 0.001$, with $\eta^2 = 0.224$. The reported aggregate results support overall differences among the exposure groups, but individual Tukey-adjusted pairwise comparisons were not presented and therefore were not attributed to specific group pairs.

Table 4. Electrochemical Responses and Serum Malondialdehyde by Occupational Exposure Duration

Measurement	<5 years (n = 22)	5–10 years (n = 35)	>10 years (n = 28)	F (2,82)	p-value	η^2
Serum MDA, μmol/L	2.81 ± 0.76	3.52 ± 0.91	4.35 ± 0.98	18.42	<0.001	0.310
Uric-acid-assigned peak current, μA	11.65 ± 2.10	14.78 ± 2.65	17.12 ± 2.84	23.15	<0.001	0.361
Ascorbic-acid-assigned peak current, μA	9.82 ± 1.54	8.41 ± 1.72	7.42 ± 1.88	11.84	<0.001	0.224

Data are presented as mean ± standard deviation. MDA: malondialdehyde; η^2: eta squared. Effect sizes were calculated from the reported F-statistics and degrees of freedom. The analyses were unadjusted for demographic, clinical, behavioural, dietary, renal, and occupation-specific confounders.

Overall, the findings demonstrated measurable electrochemical signals at the reported oxidation potentials and statistically significant relationships between their peak currents, serum malondialdehyde concentrations, and occupational exposure duration. The largest exposure-group effect was observed for the uric-acid-assigned peak current, followed by serum malondialdehyde and the ascorbic-acid-assigned peak current. Because the study did not include direct analyte-specific reference assays, calibration data, or adjusted multivariable analyses, these results should be interpreted as preliminary electrochemical and biological associations rather than confirmation of analyte accuracy, diagnostic performance, or an independent effect of occupational exposure.

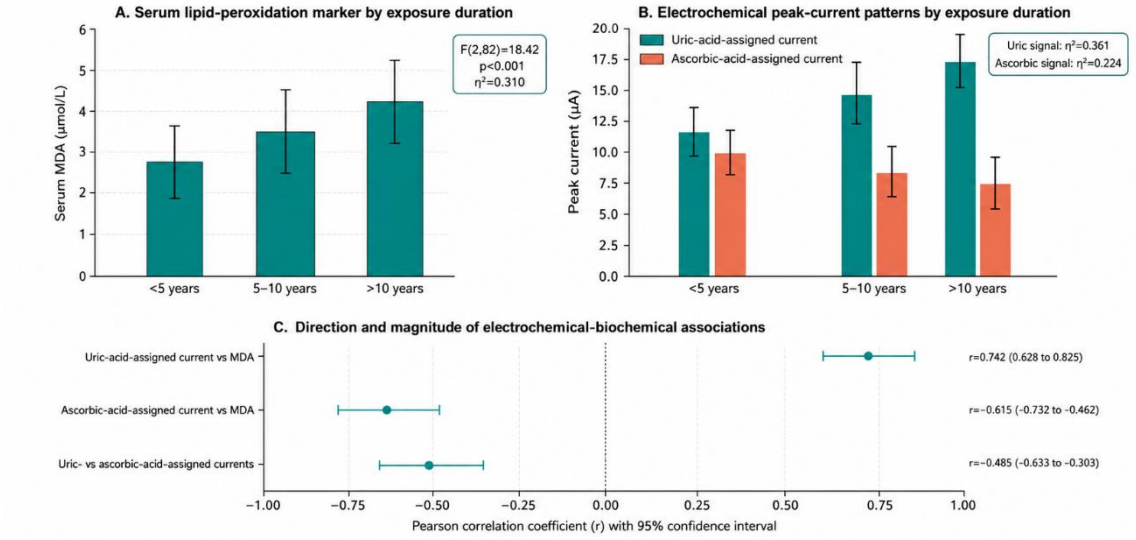

Figure legend: Panel A presents mean serum malondialdehyde concentrations across occupational exposure-duration categories. Panel B presents the mean peak currents provisionally assigned to uric acid and ascorbic acid, with error bars representing standard deviations. Panel C summarizes Pearson correlation coefficients with 95% confidence intervals for the relationships

between the electrochemical peak currents and serum malondialdehyde. MDA, malondialdehyde; SD, standard deviation. Serum MDA increased progressively from 2.81 ± 0.76 µmol/L among participants with less than 5 years of exposure to 4.35 ± 0.98 µmol/L among those with more than 10 years, with a large exposure-group effect ($\eta^2 = 0.310$). The uric-acid-assigned peak current similarly increased from 11.65 ± 2.10 to 17.12 ± 2.84 µA and demonstrated the largest group effect ($\eta^2 = 0.361$), whereas the ascorbic-acid-assigned peak current decreased from 9.82 ± 1.54 to 7.42 ± 1.88 µA ($\eta^2 = 0.224$). The uric-acid-assigned current was positively correlated with MDA (r = 0.742; 95% CI, 0.628–0.825), while the ascorbic-acid-assigned current was inversely correlated with MDA (r = −0.615; 95% CI, −0.732 to −0.462). These patterns represent preliminary electrochemical and biological associations rather than independently confirmed analyte concentrations or diagnostic performance.

DISCUSSION

This cross-sectional analytical study found that a neem-leaf-extract-modified glassy carbon electrode generated two distinguishable serum oxidation signals provisionally assigned to uric acid and ascorbic acid. The uric-acid-assigned peak current was positively associated with serum malondialdehyde, whereas the ascorbic-acid-assigned peak current was inversely associated with malondialdehyde. Serum malondialdehyde and the uric-acid-assigned current increased across categories of longer occupational exposure duration, while the ascorbic-acid-assigned current decreased. These findings indicate that the experimental electrode responded to electroactive components of serum in patterns associated with lipid peroxidation and reported exposure duration. They do not, however, establish analyte-specific accuracy, diagnostic classification, or clinical point-of-care performance.

Electrochemical techniques have increasingly been investigated for biological and clinical measurements because they can detect redox-active compounds using comparatively small sample volumes and may eventually be incorporated into compact analytical platforms. Nanostructured metal oxides, phenolic materials, and microfluidic electrochemical configurations have been used to improve surface activity, electron transfer, and signal generation in complex samples (1–4). The present findings are broadly compatible with this analytical principle because the neem-extract-modified surface produced separable anodic responses within the applied potential range. Nevertheless, the current study did not compare the modified electrode with an unmodified electrode, chemically characterize the neem extract, or quantify the contribution of individual polyphenols. It therefore cannot determine whether the observed responses resulted from specific neem-derived compounds, general surface modification, altered electron-transfer kinetics, or interactions with other serum constituents.

Natural phenolic compounds contain redox-active functional groups that may participate in electron-transfer reactions at electrode surfaces. Previous electrochemical studies involving catechin and other naturally occurring phenolic antioxidants have demonstrated that plant-derived compounds can produce measurable oxidation responses and can be incorporated into analytical sensing approaches (4–7). The present study extends this general concept to an aqueous neem leaf extract used as a mixed electrode-modifying material. However, the extract was not standardized according to total phenolic content, chromatographic composition, extraction yield, or batch-to-batch variation. Consequently, the findings support further investigation of neem extract as an experimental electrode modifier but do not identify a specific polyphenolic mechanism or establish reproducible fabrication performance.

The positive relationship between the uric-acid-assigned peak current and malondialdehyde may reflect an association between the measured electrochemical response and the broader biological environment accompanying lipid peroxidation. Uric acid contributes to extracellular antioxidant capacity but is also influenced by purine metabolism, renal clearance, diet, inflammation, obesity, cardiovascular risk, and metabolic disease. A larger current at the assigned potential therefore cannot be interpreted solely as compensatory antioxidant upregulation. It could also reflect variation in uric acid concentration, interference from compounds oxidized at a similar potential, or matrix-dependent changes in electrode response. Direct comparison with an accepted enzymatic or chromatographic uric acid assay, together with standard-addition and interference experiments, would be required to confirm the identity and analytical meaning of this signal.

The inverse association between the ascorbic-acid-assigned current and malondialdehyde is biologically plausible because ascorbic acid participates in non-enzymatic antioxidant defence and may be consumed during reactive-species scavenging. Electrochemical approaches have previously been used to investigate antioxidant activity in biological fluids and other complex matrices (2,5,8,9). Nevertheless, serum ascorbic acid is strongly influenced by dietary intake, supplement use, specimen handling, storage, oxidation after collection, smoking, and metabolic characteristics. Although individuals taking antioxidant supplements were excluded, these additional determinants were not measured. Furthermore, the signal was not compared with an analyte-specific laboratory assay. The observed inverse correlation should therefore be interpreted as an association involving a provisionally assigned oxidation response rather than direct evidence of ascorbic acid depletion.

The progressive differences across occupational exposure-duration groups were notable. Participants reporting more than 10 years of exposure had higher mean malondialdehyde and uric-acid-assigned peak currents and lower ascorbic-acid-assigned peak currents than those reporting shorter exposure. The corresponding overall effect sizes were large for the uric-acid-assigned signal and malondialdehyde and moderate-to-large for the ascorbic-acid-assigned signal. These patterns are consistent with increasing oxidative-stress-related differences across the reported duration categories. However, duration of occupational exposure was used as an indirect proxy and did not quantify pollutant type, dose, intensity, workplace controls, personal protective equipment, smoking, residential exposure, or biological toxicant burden. Participants with longer exposure may also have differed in age, occupation, health status, renal function, diet, medication use, and socioeconomic conditions. The unadjusted group differences consequently cannot be attributed independently or causally to industrial exposure.

The correlation between the electrochemical responses and serum malondialdehyde provides evidence of biological association but not analytical validation. Malondialdehyde represents lipid peroxidation and is not a reference measurement for uric acid or ascorbic acid. In addition, the thiobarbituric acid reactive substances method may detect substances other than malondialdehyde and can be influenced by specimen preparation and assay conditions. Formal validation of the electrochemical signals would require direct analyte-specific comparators, calibration curves, linearity assessment, limits of detection and quantification, within-run and between-run precision, electrode-to-electrode reproducibility, recovery studies, selectivity testing, stability analysis, and evaluation of matrix interference. Similar requirements apply broadly to electrochemical sensors intended for biological measurement or future point-of-care translation (1–4,8).

The study had several strengths. It evaluated authentic human serum samples rather than relying exclusively on prepared standard solutions, included participants with varying reported durations of industrial exposure, and compared electrochemical responses with an independently measured indicator of lipid peroxidation. The two assigned signals demonstrated different directional relationships with malondialdehyde and exposure duration, providing a coherent preliminary pattern for future investigation. The sample size also permitted reasonably precise estimates of the reported correlations, with confidence intervals that excluded null associations.

The principal limitations were substantial. The convenience sample was obtained from an imprecisely defined regional setting, limiting representativeness. No unexposed or clinically characterized control group was included. The neem extract was not botanically and chemically standardized, and essential electrode-fabrication and voltammetric parameters were incompletely reported. Peak identities were not confirmed through analyte spiking, standard addition, chromatographic comparison, or direct biochemical assays. No calibration, analytical recovery, interference, antifouling, repeatability, reproducibility, or storage-stability results were available. Important determinants of uric acid, ascorbic acid, and malondialdehyde were not measured or adjusted for, and the cross-sectional design prevented assessment of temporal or predictive relationships. The exposure categories were based on duration rather than quantified toxicant dose, while the absence of independent validation increased the

possibility that the observed associations were specific to the present sample and measurement conditions.

Further investigation should therefore proceed as staged analytical development rather than immediate clinical implementation. The neem extract and modified electrode should first be standardized and chemically characterized. The assigned signals should then be confirmed using pure standards, spiked serum, direct reference assays, and systematic interference testing. Calibration, precision, recovery, stability, and electrode-batch reproducibility should be evaluated before biological interpretation. Subsequent studies could compare exposed and minimally exposed populations, measure specific environmental toxicants and relevant confounders, and use multivariable models to assess independent associations. Only after satisfactory analytical validation should the platform be evaluated prospectively for diagnostic discrimination, field usability, cost, and potential point-of-care application.

CONCLUSION

The neem-leaf-extract-modified electrode generated two distinguishable serum oxidation signals that were provisionally assigned to uric acid and ascorbic acid and demonstrated opposing associations with serum malondialdehyde and occupational exposure duration. The findings support the feasibility of further investigating neem extract as an experimental electrode-modifying material for oxidative-stress-related electrochemical analysis. However, the study did not confirm analyte identity, analytical accuracy, diagnostic performance, or point-of-care suitability; comprehensive physicochemical characterization and formal analytical validation are required before clinical interpretation or implementation.

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