Effect of Nature-Based Interventions on Mental Health and Inflammation Markers in Women Victims of Violence Residing in the City of Bogotá

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1. Executive Summary

Gender-based violence in Colombia represents a social challenge of alarming proportions, with profound effects on the mental health of women and on the social fabric as a whole. This form of violence, rooted in power imbalances and discriminatory cultural norms, not only perpetuates cycles of abuse and trauma but also creates conditions favorable to the development of violent behaviors in future generations. The problem extends beyond the individual level, affecting social cohesion and overall well-being. Furthermore, gender-based violence leaves victims in a state of emotional and psychological vulnerability, contributing to a higher incidence of anxiety and mental disorders in the population.

This project addresses both the psychological implications and the biological markers of violence against women. It seeks to fill a gap in scientific knowledge by exploring the relationship between violence, alterations in pro-inflammatory cytokines, and their impact on the immunological and mental health of a group of Colombian women affected by gender-based violence.

The intervention combined nature immersion therapy sessions conducted by trained nature therapy practitioners with horticulture activities supported by CityHuertas, delivered over four months through 14 weekly sessions alternating between both modalities. This combination of interventions is expected to not only address the immediate consequences of violence but also promote the integral well-being of affected women, establishing a replicable intervention model for other communities. Two assessments were conducted at the beginning and at the end of the intervention: psychological components were evaluated using the Davidson Trauma Scale, the Beck Anxiety Inventory, and the Beck Depression Inventory-II, along with physiological determination of cortisol, pro-inflammatory cytokines, and immune cells.

The articulation of the project with public research and innovation policy is evidenced by its alignment with the Mission for Science for Peace and the Mission for Health Sovereignty and Social Well-being. From a peace perspective, the research contributes to knowledge about the consequences of gender-based violence and to the development of strategies for peacebuilding in households. From a health standpoint, the project strengthens understanding of the implications of violence on women's mental health and contributes to the development of more effective and sustainable interventions.

In conclusion, the research addresses a significant social problem while promoting scientific and technological innovation in the field of mental health and gender-based violence. The results of this project are expected to contribute significantly to intervention and support for women affected by violence, as well as to the construction of more resilient and healthy communities in Colombia.

Keywords: gender, women, nature, intervention, violence, chronic inflammation, mental health.

2. Alignment with Public Policy and/or Research Lines

This project aligns with public policy through specific objective 1: increasing scientific vocations in children and youth, training in science, technology and innovation (STI), and linking related human capital to the labor market to close human talent gaps, strengthen STI human capital, and increase the insertion and demand of doctoral graduates in the productive sector. This is achieved through the training of the young researcher in the area of Science, Technology and Innovation, as well as the strengthening of scientific knowledge through the doctoral researcher. Through specific objective 2, the project addresses improving the capacity for generating scientific and technological knowledge and the capabilities of research groups and support entities, to increase the quality and impact of knowledge on Colombian society. The project promotes open science through interventions with the population affected by gender-based violence, including labor training sessions in plant cover management such as activities related to the restoration and maintenance of young trees, gardening, plant propagation, and agroecological gardens, thereby promoting the empowerment of women and strengthening their capacities and skills for their integral and independent development.

This project is also framed within the research lines of the National System of Science, Technology and Innovation through the following routes: environmental health, contributing updated knowledge on the environment-health relationship to improve environmental quality for population well-being; health systems and policies, contributing scientific evidence for understanding population health needs and decision-making in policies and programs; and health equity and migration, generating scientific evidence on conditions that create gaps and affect the health of population groups living in disadvantaged contexts.

The project additionally aligns with the Mission for Science for Peace, specifically with the objective of fostering new citizenships and a culture of peace that guarantee the reconstruction of the social fabric in communities across the country's diverse regions and territories. Within this framework, the following elements are recognized: techniques and tools for identifying the impacts of violence on the mental health of the Colombian population; psychosocial care services with differential approaches that holistically promote mental health; ecosystems of thought to promote a culture of peace from the diversity of citizenships; and research integrating science and art to consolidate peace narratives.

3. Identification and Description of the Problem

Gender-based violence represents a central problem with devastating repercussions for the generation of societies marked by violence, anxiety, and family disintegration. This form of violence, rooted in power imbalances and discriminatory cultural norms, not only perpetuates cycles of abuse and trauma but also generates a conducive environment for the development of violent behaviors in future generations. Households affected by this issue often lack models of peaceful conflict resolution, increasing the likelihood that children

and young people will internalize violence as a legitimate means of resolving disputes. Furthermore, gender-based violence, which includes domestic violence, attempted femicide, armed conflict, trafficking, abuse, and sexual exploitation, among others, can leave individuals in a state of emotional and psychological vulnerability, contributing to a higher incidence of anxiety and mental disorders in the population.

The scope of gender-based violence transcends the individual sphere, extending as a dark shadow over the social fabric of the country and the achievement of total peace. This corrosive phenomenon manifests in various spheres of community life, from the deterioration of interpersonal trust to the destabilization of family structures. In communities affected by high rates of domestic violence, an increase in distrust toward authorities and a reluctance to seek help due to the stigma associated with victimization is observed. Likewise, gender-based violence can perpetuate an intergenerational cycle of inequality and marginalization, eroding social cohesion as future generations grow up in an environment marked by trauma and insecurity. This public health crisis not only affects the direct victims of violence but also imposes a significant burden on healthcare resources and justice systems, exacerbating social and economic inequalities. Consequently, it is essential to address gender-based violence not only as an individual problem but as a systemic threat requiring a comprehensive and coordinated response at the community and governmental levels.

It is essential to recognize the severity of this problem and act decisively to mitigate its devastating effects, both at the individual level and on the social fabric as a whole. According to Bulletin 108 of the Attorney General's Office, in the first months of 2024, 84 alerts were generated for domestic or partner violence, and at least 21 women were victims of femicide during January, in 11 of which they were allegedly killed by their partner or ex-partner, with most cases committed using bladed or sharp weapons. According to the report on Gender-Based Violence between January 1 and July 31, 2023, Colombia records more than one femicide per day. Data from the Criminal Justice System indicate at least 301 femicides at the national level, with Bogotá (59 cases), Valle del Cauca (36), Antioquia (35), Tolima (16), and Santander (16) showing the highest prevalence. Regarding domestic violence in 2023, more than 203 cases against women were reported per day; between January and July 2023, 60,469 complaints of domestic violence were filed nationally, of which 71% (42,914) involved female victims.

Additionally, beyond deaths, injuries, and disabilities resulting from domestic violence, the negative effects on the emotional health of the female victim and the possible repercussion on the development of chronic diseases must be considered. Recent scientific research is allowing us to understand how permanent emotional and behavioral changes can lead to cellular and biochemical changes related to the phenomenon of inflammation, which in turn cause chronic diseases such as heart disease and metabolic syndrome, a precursor to diabetes, among others.

Despite the existence of programs and public policies focused on gender equity, protection, restitution of rights, and the empowerment of women, the current situation poses a significant challenge in the comprehensive evaluation and approach to mental health

problems resulting from domestic violence. This underscores the need for a deep understanding of the biological effect of stress-reducing interventions that negatively affect women and their families.

Therefore, the project has as its fundamental objective to establish the effect of nature-based interventions on indicators of post-traumatic stress, anxiety, depression, coping, and cellular markers of inflammation in women victims of violence. Through rigorous and multidisciplinary research, the aim is not only to understand the biological impact of these interventions but also to provide scientific evidence supporting the implementation of alternative and holistic therapies in addressing gender-based violence.

4. Background

In 2023, 848 alerts were issued for femicide risk and 21 women were victims of this crime in January alone. In Bogotá, the situation is equally concerning: between January 2022 and August 2023, the number of victims of domestic violence increased by 5%, from 26,809 to 28,131. The localities with the highest incidence were Kennedy (13%), Suba and Ciudad Bolívar (12%), Bosa (11%), and Engativá (9%). In terms of types of violence, psychological violence was the most prevalent (84%), followed by physical (53%), economic (4%), sexual (3%), and abandonment (1%). It was reported that 74% of women attended were between 27 and 59 years of age.

Numerous studies describe the therapeutic benefits of exposure to nature and its positive effects on mental health. It has been shown that immersion in natural spaces reduces stress and anxiety and improves sleep quality. Previous research suggests that the presence of green areas in urban environments, including urban afforestation and ecological restoration, has been associated with improved quality of life and psychological well-being.

Exposure to violence can affect levels of stress, anxiety, and physiological biomarkers, which can be reflected in cytokines, key mediators of the immune response. Several investigations have demonstrated that people who have been victims of violence present elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNF-alpha). While normal levels are essential for defense against infections and pathogens, elevated levels of pro-inflammatory cytokines can be detrimental to health, affecting the immune response and increasing susceptibility to infections and chronic diseases such as diabetes and heart disease, among others. Chronic exposure to violence can generate a state of systemic inflammation, characterized by elevated levels of pro-inflammatory cytokines that can affect the functioning of the central nervous system, contributing to symptoms such as anxiety and sleep problems.

Therefore, gender-based violence not only has an emotional and social impact but can also have negative consequences for the physical health of women. It is important to understand these relationships to develop intervention strategies that address not only the psychological but also the physical consequences of violence. These antecedents support the proposed research by providing evidence on the relationship between nature, mental health, and the immune response.

5. Justification

This research focuses on the mental health consequences and cellular markers of inflammation caused by violence against women, a social problem that affects both psychological well-being and the immune system. We seek to fill a gap in knowledge by exploring the relationship between violence, alterations in pro-inflammatory cytokines, and their impact on the immunological and mental health of affected women.

Stressful events normally promote short-term neuroendocrine and immunological changes that help meet environmental demands. However, constant stress and anxiety can chronically overload adaptive biological systems, increasing vulnerability to physiological and behavioral pathologies. The connection between traumatic experiences and the immune response constitutes an emerging area of research, and this study seeks to provide relevant data to strengthen understanding of these complex mechanisms.

From a comprehensive perspective on domestic violence, the research proposes to improve psychosocial care services and support for women victims of violence through the integration of natural interventions with the evaluation of psychological and biological markers. This approach has the potential to contribute to more effective strategies for the prevention and treatment of long-term consequences. Furthermore, the combination of nature immersion therapies and horticulture activities presents a novel approach with practical applications in mental health and integral well-being, establishing a model of tangible and replicable interventions in other communities affected by violence.

In summary, this research not only addresses two important social problems (gender-based violence and mental health) and innovates in the scientific exploration of nature-based solutions for human health, but also contributes significantly to scientific knowledge, introduces technologically innovative approaches, and has a tangible impact on the care and support of women affected by violence.

6. Articulation with Research Policy Missions

The research project on the effects of violence against women on mental health and physiological markers articulates with the research routes of the Innovation and Research Policy guided by the Ministry of Science, Technology and Innovation missions as follows:

Mission 1: Health Sovereignty and Social Well-being. Research route: Mental health and social well-being. The project strengthens research on the impact of gender-based violence on women's mental health and contributes to the development of more effective intervention and treatment strategies. By investigating this topic, the project contributes to the development of sustainable community-based strategies that promote social well-being and the quality of life of affected women, as well as to the construction of more resilient and healthy communities in the long term.

Mission 2: Science for Peace. Research route: Peacebuilding and citizen coexistence. The project contributes to the Mission of Science for Peace by contributing knowledge about the consequences of gender-based violence and simultaneously developing strategies for peacebuilding in households. Recognizing that gender-based violence is an important

component of urban violence, addressing this problem from a scientific perspective is fundamental to promoting peace and coexistence in cities.

7. Conceptual Framework

7.1 Definition: Gender and Violence

Violence, according to the definition of the Pan American Health Organization, is the intentional use of physical force or power, in fact or as a threat, against oneself, another person, or a group or community, that causes or has a high likelihood of resulting in injury, death, psychological damage, developmental disorders, or deprivation.

The relationship between gender and violence has been the subject of numerous studies that highlight the historical, social, and cultural connection between violence and masculinity. The manifestation of violence against women ranges from attacks on self-esteem, limitations in social relationships, and control of activities, to more extreme forms such as physical aggression and homicide.

Violence against women in the family sphere can have serious consequences for health, both physical and emotional, becoming a public health problem. The cycle of violence is structured in three phases: the accumulation of tension, the explosion or aggression, and reconciliation, marked by the aggressor's regret, who seeks forgiveness and promises to change.

7.1.2 Policy and District Programs on Gender Equity in Colombia

The update of the Public Policy on Women and Gender Equity responds to the persistence of inequities and violence affecting women throughout their lives. This policy seeks to progressively and sustainably modify the conditions of discrimination, inequality, and gender subordination in public and private spheres. The creation of the District Secretary of Women in 2012 and the District Care System in 2020 are prominent examples of district-level action to support women victims of gender-based violence.

7.2 Violence and Its Effect on Mental Health

Violence, as a traumatic and prolonged experience, exerts a significant impact on the mental health of victims. Its psychological effects manifest in various ways, among the main aspects to consider are:

Post-traumatic stress disorder (PTSD): characterized by intrusive memories, nightmares, anxiety, and intense physical reactions to situations that act as triggers.

Depression and anxiety: feelings of hopelessness, helplessness, and fear can become deeply rooted in the victim's psyche, affecting their emotional well-being and their perception of the world.

Self-esteem and self-concept: constant mistreatment and repetition of negative and devaluing messages can lead to a distorted perception of oneself, generating doubts about personal worth and the capacity to make decisions.

Sleep quality: violence contributes to sleep disorders, such as insomnia or recurrent nightmares.

Social isolation: whether imposed by the aggressor or as a result of shame and fear, this isolation can contribute to the exacerbation of mental health problems by limiting access to support networks.

The understanding of these effects is essential for the design of effective interventions that address both the psychological and physical aspects of violence. Levels of post-traumatic stress, anxiety, depression, and sleep quality caused by exposure to various factors can be measured through validated self-report questionnaires in different types of populations.

7.2.1 Post-Traumatic Stress Disorder

PTSD is a psychological disorder that can develop after a traumatic experience, such as violence against women or exposure to armed conflict. In the context of violence, victims may experience PTSD due to repeated exposure to situations of physical, emotional, or sexual abuse. This type of trauma can cause a series of characteristic symptoms: intrusive memories of the traumatic event, avoidance of associated stimuli, and negative changes in thinking and mood.

7.2.2 Resilience and Coping Strategies

Resilience, understood as the ability to adapt positively to adverse situations, is compromised due to the emotional and psychological burden generated by the traumatic experience. In victims of violence, this change can manifest as a decrease in self-confidence, greater susceptibility to daily stress, and a distorted perception of safety in their environment.

Psychosocial risks do not affect all people equally, as their effect depends in part on the coping strategies developed by each individual when faced with a potentially dangerous situation. Coping is defined as those constantly changing cognitive and behavioral efforts developed to manage specific external and/or internal demands that are evaluated as exceeding or overwhelming the individual's resources. Therefore, people who are victims of violence depend in part on the coping strategies employed, generating internal or external behaviors that allow them to manage the various situations generated by the violence to which they are exposed.

7.3 Cortisol Levels in Response to Stress

Cortisol, a fundamental glucocorticoid in humans, exerts a significant influence on brain function, behavior, and cognition. It is suggested that the diurnal rhythm of cortisol interacts with environmental factors to anticipate adaptation to stress. In situations of acute stress, activation of the hypothalamic-pituitary-adrenal (HPA) axis triggers the release of steroid hormones, such as cortisol and dehydroepiandrosterone. These hormones perform opposing regulatory functions in various physiological functions. Increased cortisol concentrations affect processes such as glucose release to facilitate the 'fight or flight' response and suppression of immune function.

Studies have revealed increases in cortisol levels in individuals who have experienced trauma. Cortisol levels are higher in those who have recently experienced the traumatic event but attenuate in people with non-recent exposure, such as adults who experienced trauma during childhood. Additionally, individuals facing continuous stress, such as unemployment, present higher cortisol levels compared to those who have experienced trauma in the past or are not currently subjected to stressful situations. Dysregulation of the HPA axis, indicated by cortisol suppression, implies that the body does not return to physiological homeostasis, thereby prolonging the stress response.

7.4 Violence and Its Effect on Cytokines

Social and environmental stressors such as a person's social environment and lifestyle can provoke an uncontrolled activation of the stress response, leading to the development of chronic systemic inflammation, which is a factor related to the development of harmful effects on cardiovascular health as well as increased mortality levels. There is currently strong evidence that inflammation is implicated in the pathogenesis of multiple health problems, particularly in the manifestations of cardiometabolic diseases including obesity, diabetes, metabolic syndrome, and strokes.

The inflammatory environment resulting from these disorders is characterized by elevated levels of pro-inflammatory markers, including C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 beta (IL-1b), interleukin-2 (IL-2), tumor growth factor (TNF-alpha), and interferon gamma (IFN-gamma), among others. Longitudinal studies have reported that PTSD caused an increase in inflammatory markers and that elevated levels of CRP and TNFRII were associated with a diagnosis of PTSD.

Research has shown that stress caused by low socioeconomic status, adversity, and life events can cause an increase in pro-inflammatory cytokines. Additionally, patients with a diagnosis of depression have significantly greater production of peripheral pro-inflammatory cytokines IL-6 and TNF-alpha.

7.5 Nature-Based Therapies

In recent years, the importance of conscious nature immersion therapies has been recognized as an effective strategy for reducing levels of stress, anxiety, and depression, and for improving physical and mental health. This approach is based on exposure to natural stimuli that generate a state of physiological relaxation and activate weakened immune functions. Forest therapy, known as Shinrin-yoku, emerged in Japan in the 1980s and has consolidated itself as an alternative, preventive, and curative medical practice.

Studies have shown that forest therapy has significant therapeutic effects on human health. An increase in cells related to the immune response, the reduction of anxiety and stress, and an improvement in mental relaxation have been observed. Nature immersion therapies have also been evaluated in different cultural contexts, showing consistent results in the reduction of psychological symptoms and an improvement in mood.

7.6 Intervention Evaluation Studies

Intervention evaluation studies are a type of research conducted to evaluate the impact or effectiveness of a specific intervention in a group of subjects or in a given population. In this type of study, a planned and controlled intervention is implemented, which may be a program, treatment, policy, or practice, with the objective of modifying a behavior, improving a health condition, or reducing the risk of disease, among other outcomes. These studies generally use experimental or quasi-experimental designs, such as randomized clinical trials, cohort studies, or case-control studies, to determine the efficacy and impact of the intervention on the desired outcomes.

8. Target Population

The target population will be women victims of gender-based violence. The group will consider differential approaches as well as intersectionality through the inclusion of women victims of gender-based violence including domestic violence, attempted femicide, armed conflict, trafficking, abuse, and sexual exploitation, among others, with diverse sexual identities and orientations: heterosexual women as well as those belonging to the LGBTIQ+ group. The inclusion of women in extreme vulnerability due to conditions of poverty is also considered.

9. Objectives

9.1 General Objective

To evaluate the effect of nature-based interventions on mental health and inflammation markers in women victims of violence residing in the city of Bogotá: an intervention study 2024-2025.

9.2 Specific Objectives

1. To determine the effect of nature-based interventions on post-traumatic stress, levels of anxiety, depression, and coping capacity in women victims of violence.

2. To evaluate the effect of nature-based interventions on hair cortisol concentration, pro-inflammatory cytokines, and immune cells in women victims of violence.

10. Methodology

Quasi-experimental non-probabilistic intervention study design (before and after). In this type of design, participants were selected intentionally and not at random, and were assigned to a single nature-based intervention group. Women victims of violence were chosen for specific reasons that may have been related to their availability.

10.1 Sample and Sampling

A non-probabilistic sequential sample of volunteer women victims of violence residing in the city of Bogotá was conducted, recruited with the support of the Centro de Promoción y Cultura of the Kennedy locality. To estimate the minimum sample size, the prevalence of post-traumatic stress reported in the population displaced by political violence in Colombia (9.9%) was used. A precision of 10% and a confidence level of 95% were considered, estimating a minimum sample size of 30 participants.

10.2 Population and Eligibility Criteria

Women victims of gender-based violence were considered, including domestic violence, attempted femicide, armed conflict, trafficking, abuse, and sexual exploitation, identified by the Centro de Promoción y Cultura of the Kennedy locality and recently integrated into the recruitment process for the present project.

Inclusion Criteria: women victims of domestic violence, attempted femicide, armed conflict, trafficking, abuse, and sexual exploitation, among others. Residents of the city of Bogotá. Women victims of violence with diverse sexual identities and orientations were included, as well as women victims of violence living in extreme vulnerability due to conditions of poverty.

Exclusion Criteria: women who had participated in any other type of psychosocial intervention in a program developed by any governmental, private, or non-profit organization prior to recruitment into the present study.

10.3 Selection of the Intervention Site

The selection of natural environments for the intervention was carried out considering the following criteria: tree groups, surface area, species density, presence of undergrowth, accessibility of the natural area, presence of fauna, water bodies, and degree of anthropic intervention. Nature immersion sessions took place in green spaces familiar to participants within their residential area, including a neighborhood park, the Humedal de la Vaca wetland, Parque Timiza, and a final session at Parque EntreNubes. Horticulture sessions were conducted in a rooftop garden with three raised garden beds built at a facility of the Centro de Promoción y Cultura of the Kennedy locality, with technical support from CityHuertas.

10.4 Development and Follow-up of the Intervention

The intervention lasted four months and consisted of 14 weekly sessions alternating between two modalities. The first modality consisted of nature immersion therapy sessions (Shinrin-yoku) conducted by trained nature therapy practitioners, structured in four moments: reduction of pace, stimulation of the senses, reconnection, and sharing. These sessions included short and slow walks with moments designed to awaken the senses and foster conscious connection with nature. The second modality consisted of horticulture activities with technical support from CityHuertas, involving soil preparation, sowing, transplanting, irrigation, and harvesting of vegetables and aromatic plants.

Simultaneously, an initial and final assessment was carried out using the self-administered psychometric scales detailed in the research, aimed at determining mental health in relation to post-traumatic stress, anxiety, depression, and coping capacity. Additionally, a measurement of cytokine concentrations and the determination of the proportion of immune cells in the study population were performed at the beginning (after the recruitment process was completed) and at the end of the four months of intervention.

10.5 Psychometric Tests

Standardized self-administered psychometric scales were applied at the beginning and end of the intervention to measure post-traumatic stress, anxiety, depression, and coping.

a) Davidson Trauma Scale (DTS): composed of 17 items that evaluate the frequency and severity of PTSD symptoms. Response format provides 5 options for frequency (from 0 = never to 4 = every day) and 5 for severity (from 0 = none to 4 = extremely high). The total score ranges from 0 to 136, where higher scores indicate a greater incidence of PTSD, with an overall internal consistency of alpha = 0.99.

b) Beck Anxiety Inventory (BAI): a 21-item self-administered instrument. Evaluates the severity of symptomatic and behavioral categories of agoraphobia and panic. The score ranges from 0 to 63, with higher scores indicating greater anxiety symptomatology. Final scores are classified as: minimal anxiety (0 to 7 points), mild anxiety (8 to 15 points), moderate anxiety (16 to 25 points), and severe anxiety (26 to 63 points).

c) Beck Depression Inventory-II (BDI-2): a 21-item self-administered instrument. In each item, four alternatives ordered from least to most severe are chosen. The score ranges from 0 to 63. Final scores are classified as: no depression (0 to 9 points), mild depression (10 to 18 points), moderate depression (19 to 29 points), and severe depression (30 to 63 points).

d) Coping Strategies Inventory (CSI): an instrument for evaluating coping strategies applied worldwide, corresponding to a Spanish adaptation of the Coping Strategies Inventory. The instrument presents a hierarchical structure in which the person answers each item on a four-point Likert scale, where 0 = not at all, 1 = a little, 2 = quite a bit, 3 = a lot, and 4 = completely.

10.6 Biological Sample Collection

Peripheral blood samples were collected from each participant at two time points: at the beginning of the study (after completing the recruitment process) and at the end of the 4-month intervention period. A total of 4 Vacutainer tubes with anticoagulant were collected per participant at each sampling, with approximately 4 mL of blood collected in each tube. Blood samples were taken by qualified and experienced Instituto Nacional de Salud personnel at a private and adequate space provided by the Centro de Promoción y Cultura of the Kennedy locality. Afterwards, samples were transported to the Environmental and Occupational Health Group laboratory.

Additionally, hair samples were collected from the posterior vertex of the scalp. The proximal 6 cm segments of hair were weighed to obtain 15 mg. These samples were also collected by qualified INS personnel at the same facility and stored at room temperature until analysis.

10.7 Extraction and Freezing of Peripheral Blood Mononuclear Cells

The extraction of peripheral blood mononuclear cells (PBMCs) was performed on the same day as blood sample collection, through a sedimentation separation using Ficoll-Paque as a gradient medium for the separation of cellular fractions. The integrity of the PBMCs was ensured through RPMI medium supplemented with fetal bovine serum during the extraction procedure. Ficoll-Paque was added in a 1:3 ratio relative to the amount of packed

cells obtained. Two washes of the leukocyte layer were performed with phosphate buffer 1X at pH 7, supplemented with 1% fetal bovine serum. The freezing medium consisted of 50% RPMI 1640, 40% fetal bovine serum, and 10% dimethyl sulfoxide (DMSO). PBMCs were maintained at minus 80°C until the time of analysis.

10.8 Determination of Immune Cell Proportions

The proportion of T cells, B cells, and monocytes was determined through the design of a panel with specific antibodies using the flow cytometry technique. Titration of each antibody was performed to determine the optimal concentration. Compensation of the equipment was carried out according to the acquired fluorometers and each sample was labeled with monoclonal antibodies for 15 minutes at room temperature in darkness, for reading in the FACSMelody BD cytometer. Each measurement involved a positive control and a negative control. Data analysis was performed using FlowJo software.

10.9 Determination of Inflammatory Markers

Peripheral blood samples were collected using Vacutainer tubes with anticoagulant for serum extraction. Samples were analyzed using a multiplex assay using the Cytokine 10-Plex Human Panel, specifically designed to quantify the following cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-gamma), interleukin-1 beta (IL-1b), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNF-alpha). In total, samples corresponding to the beginning and end of the intervention were analyzed. The concentration of cytokines was expressed in pg/mL.

10.10 Determination of Hair Cortisol

Samples were washed twice for 3 minutes using 5 mL of isopropanol (reagent grade, 99%) to eliminate contamination from sweat and sebum. After drying, weights were adjusted, recovering a minimum of 10 mg from each sample. Samples were analyzed by immunoassay using the Abcam ELISA Kit (Product Code: ab108665), following the manufacturer's instructions for precise detection of cortisol concentration in hair. Cortisol was extracted by pulverizing the hair, adding 500 uL of 100% methanol, incubating at 60°C for 30 minutes, centrifuging at 10,000 x g for 10 minutes, and transferring the supernatant to a new Eppendorf tube. The methanol was evaporated at room temperature and the residue was reconstituted with 500 uL of PBS buffer 1X. Each hair sample was analyzed in triplicate to evaluate variability and ensure precision of results.

10.11 Statistical Analysis

The normality of continuous variables was evaluated by the Shapiro-Wilk test. Results are presented as medians with interquartile ranges for non-normally distributed data and as arithmetic means with standard deviations for normally distributed data, while categorical variables were expressed as absolute and relative frequencies.

Comparisons between baseline and post-intervention values were performed using the Wilcoxon signed-rank test. Change scores for psychological parameters were calculated as

post-intervention minus baseline values. Correlations between changes in psychological variables were assessed using Spearman's rank correlation test.

To explore potential relationships between psychological outcomes and immunological biomarkers associated with the intervention, principal component analysis (PCA) was conducted separately for soluble inflammatory markers and cellular immune parameters to reduce collinearity and identify coordinated biological patterns. Cytokine concentrations were log-transformed to reduce right skewness and stabilize variance. Within-individual change scores were calculated as the difference between post-intervention and baseline values and were subsequently standardized prior to PCA. The first two cytokine principal components were retained based on the proportion of explained variance and interpretability of their loading structure. For cellular immune subsets, percentages were converted to proportions (0 to 1) and transformed using the logit function to account for their bounded distribution. Within-individual change scores were calculated as delta logit (cell proportion) and standardized prior to PCA. The first principal component was retained as a summary index of coordinated cellular immune changes.

A multivariable generalized linear regression model was constructed to evaluate the association between immunological changes and global psychological improvement. The dependent variable was a standardized global psychological change index derived from the combined changes in depressive symptoms, anxiety, and post-traumatic stress symptoms. Independent variables included the first two cytokine principal components and the first cellular immune principal component. The model was adjusted for age, duration of violence exposure, and lifetime exposure to either a single or multiple forms of violence. Model assumptions were evaluated through inspection of residual distributions. A p value less than 0.05 was considered statistically significant. All analyses were conducted in R (version 4.5.1), and figures were generated using R (version 4.5.1) and GraphPad Prism (version 8.0.2).

11. Bias Control

Information bias: in order to minimize memory biases during the collection of required information, open-ended questions were not used in the questionnaires used in the study.

Confounding bias: to control the possible confounding effect of variables such as age, sex, and smoking habits in estimating the association between exposure to the intervention and psychological and biological biomarkers, each analysis was adjusted for these variables. This allowed for a more precise evaluation of the impact of the intervention, minimizing the influence of external factors on the results.

Measurement bias: standardized measurement protocols were strictly followed to reduce measurement biases. Rigorous quality control procedures were implemented to ensure accurate and reliable data collection. This included adequate training of personnel responsible for data collection, regular calibration of measurement instruments, and periodic verification of the consistency of collected data.

Interpretation bias: to minimize biases in the interpretation of study results, clear and predefined criteria were established for data analysis and interpretation. This included the

development of a detailed analysis plan specifying how different scenarios and unexpected results would be addressed, as well as review by a committee of experts to ensure objectivity in the interpretation of findings.

12. Ethical Considerations

Declaration of Helsinki: This protocol has considered the aspects indicated by the Declaration of Helsinki on research ethics. In particular, it has been considered that the conduct of this research promotes improving preventive and therapeutic interventions in the management of post-traumatic stress, anxiety, and sleep quality in women victims of violence. This intervention was designed to be safe, accessible, and of quality. The research upholds respect for study participants and their individual rights, where their interests take precedence over those of the research itself. Workers who decide not to participate or continue in the study may do so at any time.

Resolution 8430 of 1993 of the Ministry of Health, which establishes the academic, technical, and administrative standards for health research, classifies this research as minimal risk.

Prior to the start of data collection, individuals were informed of the objectives, type of study and follow-up, its importance, and the benefits of participating. Each individual was provided with an informed consent form that they must sign if they voluntarily agree to participate in the study, before answering the individual survey questions and before biological sample collection. The project was presented to the Ethics and Research Methodologies Committee (CEMIN) of the Instituto Nacional de Salud.

13. Use of 4.0 Technologies

The selection of natural environments for the intervention was performed using civil-use spatial technology that allowed for the capture of geospatial data to analyze the physical conditions of each natural environment in Bogotá. This system results in a detailed 3D representation of the urban landscape. Tools used included GRASS GIS, QGIS, R, SIGAU, Arbolapp, and Whitebox, ensuring coverage and reliability of results.

14. Sustainability of the Project

The relevance of the nature-based intervention project for women victims of violence extends beyond its immediate impact and contemplates the need to consider its long-term sustainability and expansion to other vulnerable population groups. The sustainability of the proposal lies in the results obtained and their dissemination, responding to the hypothesis that nature-based interventions will have a positive effect on the mental health and well-being of women victims of violence. To guarantee long-term sustainability and expansion of the project, the following actions are proposed:

1. Establishing strategic alliances with local and governmental organizations to guarantee the continuity and long-term support of nature-based interventions for women victims of violence.

2. Proposing in health centers the development of a training plan for health professionals and social workers on the implementation and follow-up of interventions, ensuring their sustainability over time.

3. Seeking and establishing long-term financing mechanisms to ensure the continuity of the interventions, exploring options such as government funds, private donations, and collaborations with international organizations.

4. Encouraging community participation in the design, implementation, and follow-up of interventions, promoting local ownership and empowerment of women victims of violence.

5. Conducting awareness and continuous education campaigns on the importance of addressing gender-based violence and promoting mental health in the community.

15. Expected Results and Impacts

1. Socioeconomic development: the project is expected to promote the development of skills and acquisition of knowledge among participating women, which will allow them to start businesses or access employment that improves their socioeconomic conditions, driving economic inclusion and financial autonomy.

2. Improvement in mental health: specifically, a decrease in anxiety, depression, and post-traumatic stress is expected, along with an improvement in coping strategies. This improvement in mental health will be fundamental for their overall well-being and their ability to face daily challenges.

3. Impact on physical health: the project is expected to modify the concentrations of cellular biomarkers of inflammation and their relationship with the proportion of immune cells, which will contribute significantly to improving the physical health of participants.

4. Advancement of scientific knowledge: the project will generate important scientific advances by investigating the beneficial effects of nature-based interventions on human health, both at the psychological and biological levels.

5. Environmental impact: the project will seek to raise awareness in the population about the importance of conserving green spaces in urban areas and will promote the use of sustainable transportation.

16. Public Dissemination Strategy

Presentation of results at a national scientific event relevant to the field of public health and environmental research.

Publication of a research article in a peer-reviewed indexed journal of at least category B.

Strengthening of the Environmental and Occupational Health Research Group of the Instituto Nacional de Salud through strategic collaborations and alliances.

Training of human resources through a continuous training program aimed at the research team and personnel involved in the project.

17. Expected R&D+i Products

Research article (E1): original article as a result of the research submitted to an indexed journal of at least category B. Verification: copy of the article submission confirmation.

Participation in a scientific event (AA1): participation in a scientific event presenting the research results. Verification: certificate of participation in poster or oral modality and supporting documentation of the presented work.

Brochure (E2): development of a brochure to disseminate project results to participants. Verification: copy of the brochure and document certifying its distribution.

Participation in knowledge networks (AA2 and AA3): presentation of the experience in two network events on strengthening women's capacities. Verification: document issued by the entity certifying network articulation activities.

Digital content production (E3): generation of a video summarizing the intervention carried out with the study population. Verification: copy of multimedia content.

Young researcher training (FRH1): linking of the young researcher to the project. Verification: linkage contract of the young researcher and progress and final report of activities developed within the framework of the project.

18. Schedule

Month 1 to 2: development of field guides and training of personnel, standardization of procedures. Recruitment of study population and signing of informed consent. Initial collection of biological samples.

Month 1 to 4: first application of sociodemographic and psychological instruments. Nature-based intervention (alternating nature immersion therapy sessions and horticulture activities). Young researcher training.

Month 4: final collection of biological samples. Second application of psychological instruments.

Month 5 to 6: determination of cytokine concentrations using Luminex 200. Determination of immune cell proportions by flow cytometry. Statistical analysis of results obtained.

Month 6 to 8: preparation of the final report of results obtained. Writing of a scientific article. Participation in a scientific event. Development of brochure. Participation in knowledge networks. Production of digital content.

19. Risk Analysis

The risks specifically associated with the development of the project have been evaluated to ensure comprehensive management. The following risks have been identified:

1. Personnel to be contracted: risk of non-linkage of the young researcher due to better economic offers (medium risk). Mitigation: offer flexible schedules and allow telework when necessary.

2. Materials, supplies, and equipment: risk due to export conditions, currency fluctuations, equipment damage and maintenance, and technological failures (high and medium risk).

Mitigation: establish reliable local suppliers, diversify supply sources, and implement preventive maintenance plans.

3. Women who do not adhere to the program: voluntary withdrawal and low participation (low risk). Mitigation: flexible schedules, development of motivational indicators, and constant follow-up.

4. Non-compliance with schedule: risk of non-compliance with indicated timelines (low risk). Mitigation: monthly meetings to review progress, minutes, photographic records, and databases.

5. Cost increases: insufficient budget due to the consumer price index (high risk). Mitigation: periodic budget review, adjustments according to the CPI, and search for additional sources of financing if necessary.

6. Communication: risk of failures in internal and external communication affecting understanding of the project (low risk). Mitigation: establish clear communication channels and use project management tools.

20. Management Indicators

Development of field guides and training: guides and minutes completed / guides and minutes planned.

Recruitment of study population: informed consent folder.

Collection of initial blood samples: peripheral blood sample collection method.

First application of psychological instruments: psychological instrument database completed / planned.

Nature-based intervention: report of activities completed / report of planned activities.

Collection of final blood samples: peripheral blood sample collection method.

Second application of psychological instruments: psychological instrument database completed / planned.

Cytokine concentration determination: cytokine determination assay method.

Immune cell proportion determination: immune cell determination assay method.

Young researcher training: final report delivered / final report planned; minutes completed / minutes planned; final satisfaction report completed / satisfaction report planned.

Statistical analysis of results: results obtained / expected results.

Preparation of final report: final report delivered / final report planned.

Writing of a scientific article: article submitted / article planned.

21. Budget

The following table presents a general overview of the project budget. For more specific details, please refer to Annex 8. The budget covers personnel (plant staff and contract staff), equipment use, materials and supplies, publications, and academic events, with

contributions from the Ministry of Sciences, the Instituto Nacional de Salud, and project counterpart resources.

22. Competence and Track Record of the Institution

Instituto Nacional de Salud - Environmental and Occupational Health Group. This group conducts research activities under three lines: environmental contaminants and health effects, biomarkers for environmental contaminants, and the work environment and its effect on worker health. It has developed scientific and technical cooperation agreements with national and international institutions. It is part of the Environmental and Occupational Health NODE Colombia, participates in the National Committee on Safety and Health at Work, and currently leads the Knowledge Management, Development and Innovation Network in Safety and Health at Work.

The group has received recognition including first place in the Cástulo Rodriguez Prize 2022 from the Colombian Society of Occupational Medicine, the prize for best research idea within the Respiration 2021 Hackers competition, and an honorable mention from the Colombian Society of Occupational Medicine.