**ALCOHOL, CIGARETTE, AND POLYHERBAL MEDICINE (AGBO) CONSUMPTION MAY IMPACT NEGATIVELY ON ANTIOXIDANT STATUS OF INDIVIDUALS IN NNEWI METROPOLIS.**

**ABSTRACT:**

**Background:** Oxidative stress, an imbalance between reactive oxygen species (ROS) and antioxidants, has been implicated in various health conditions, including cardiovascular diseases, cancer, and neurodegenerative disorders. While polyherbal formulations (Agbo) are widely consumed in Nigeria for their perceived medicinal benefits, the potential impact of their use alongside alcohol and cigarettes on oxidative stress remains largely unexplored.

**Objective:** This study evaluates the effects of Agbo, alcohol, and cigarette consumption on oxidative stress markers—Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC)—among individuals in the Nnewi metropolis.

**Method:** A cross-sectional study was conducted on 102 participants divided into three groups: individuals consuming Agbo, alcohol, and cigarettes; individuals consuming only alcohol and cigarettes; and a control group. Venous blood samples were collected and analyzed for MDA and TAC levels using Thiobarbituric Acid Reactive Substances (TBARS) and Ferric Reducing Antioxidant Power (FRAP) assays, respectively.Statistical analysis was performed using SPSS version 26.

**Results:** Results showed significantly elevated MDA levels and reduced TAC levels in individuals consuming Agbo, alcohol, and cigarettes compared to the control group (p<0.05), indicating increased oxidative stress. No significant differences were observed in participants consuming only alcohol and cigarettes when compared with those consuming all three substances, suggesting that Agbo consumption does not provide notable antioxidant protection when combined with these harmful substances. Moreover, age, duration, and frequency of consumption did not significantly correlate with oxidative stress markers in various groups.

**Conclusion:** These findings highlight the potential oxidative damage associated with the combined use of Agbo, alcohol, and cigarettes. Further studies are needed to establish safe consumption guidelines and assess the long-term health implications.

**Keywords**: Oxidative stress, Polyherbal formulations, Agbo, Alcohol, Cigarette smoking, Free radicals, Malondialdehyde, Total Antioxidant Capacity, Reactive Oxygen Species (ROS), Antioxidants

**INTRODUCTION**

Studies have shown that medicinal plants, whether used singly or combined, serve as the basis for 25–50 % of currently produced drugs used in healthcare (Kalyniukova *et al*., 2021). Over the years, people across several cultures and regions have discovered the efficacy of herbal medicines in curing or managing diverse illnesses. According to a report by the WHO, about 80% of the people in developing countries rely on traditional herbal mixtures to treat different diseases. Most villages in Africa still depend solely on traditional herbal mixtures as a source of health treatments because of their beliefs and culturally acceptable indigenous knowledge, accessibility, and affordability (Okaiyeto and Oguntibeju, 2021).

Traditional medicine can either be polyherbal formulations, combining multiple herbs in a single remedy or single-herb formulations, producing medicine from a single herb. It is observed that the activities of polyherbal extracts against various pathogens are greater than single plant extracts and found to be a more effective therapy due to the synergistic effects of active phytochemicals like flavonoids, lycopene, ascorbic acid, carotenoid, etc. (Mussarat *et al.*, 2021; Onah *et al*., 2023). Moreover, these phytochemicals have antioxidant properties. Hence, polyherbal formulations have gained attention recently due to their potential to protect the human body from free radicals.

Antioxidants are molecules that can prevent, delay, or remove oxidative damage caused by free radicals (Ogbodo *et al*., 2019). Their primary function is to neutralize the excess reactive oxygen species and safeguard cells against their toxic effects, helping prevent oxidative stress (Parcheta *et al*., 2021). They work by chelating or scavenging free radicals or modulating the activities and levels of antioxidant enzymes and their reducing potential. Oxidative stress is caused when reactive oxygen species (ROS) are produced in excess, thereby destroying antioxidant enzymes, damaging biological macromolecules such as DNA and RNA, and impairing cell signaling pathways, which can lead to apoptosis or cell death (Luo *et al*., 2020; Ogbodo *et al*., 2019). Oxidative damage to DNA and cellular components caused by ROS can lead to cancer-related mutations (Di Meo, 2020). Consequently, antioxidants help protect the human body from ROS-induced damage, and consumption of natural antioxidants has been linked to a lower risk of cancer and other diseases associated with oxidative damage, which is linked to phenolic compounds and the phenolic hydroxyl group (Aladejana, 2023).

Since these polyherbal formulations are prepared using constituents that contain antioxidants, they have proven to be effective in combating the deleterious effects of oxidative stress. However, there is a need for further research to validate the efficacy and safety of these formulations, carry out more clinical studies, identify the active constituents responsible for their therapeutic properties, and standardize their preparation and dosage. This will help prevent overdosage, toxicity, or damage to vital organs that may arise from combining the wrong herbs or due to prolonged misuse. Moreover, proper research around the subject matter may provide adequate information to guide users on usage requirements, regulatory protocols, and quality control (Aladejana, 2023).

**MATERIALS AND METHODS**

**Study Design and Population**

This was a cross-sectional study designed to assess the effects of Agbo, alcohol, and cigarette on oxidative stress markers in consumers in Nnewi. The design consisted of three groups: individuals who consume Agbo, alcohol, and cigarette; individuals who consume only alcohol and cigarette; and individuals who consume none of these who serve as control. The subjects were recruited from different motor parks within Nnewi metropolis and they were age-matched across these three groups with age range of 20 – 60 years.

The inclusion criteria include individuals aged 25 to 60 who reside within Nnewi metropolis; and who consume Agbo, alcohol, or cigarettes and those who do not consume any of these substances who served as control. The exclusion criteria included individuals who live outside Nnewi and its environs; those who declined participation; involved in any form of supplementation that influences antioxidant levels; and those under 25 or over 60 years old.

**Sample Size**

G-power software version 3.1.9.4, was used to determine the sample size and power of the study. The predicted sample size of 102 participants has an error probability of 0.05 and a 95% power to detect variations in replies as small as 0.4 (effect size). Simple random sampling method was used to recruit 102 consecutive consenting adults into 3 groups of 34 participants.

**Sample Collection and Biochemical Analysis**

Five milliliters (5ml) of venous blood were aseptically drawn from each subject's ante-cubital vein using a plastic syringe and dispensed into plain tubes. Following centrifugation at 3000 rpm for 10 minutes, the serum was separated into a new plain tube using a micropipette and then stored. The samples were kept frozen at a temperature of -20 ºC until the biochemical analysis of MDA and TAC were carried out within one month of collection.

**Determination of MDA level**

MDA level was determined by the colorimetric method of Gutteridge and Wilkins, (1982).

**Estimation of total antioxidant capacity**

Total antioxidant activity was estimated by Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, 1996.

**2.3 Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS) version 26 was used for the analysis of the results. The data obtained was presented as mean ± standard deviation (SD) and was analyzed statistically using one-way analysis of variance (ANOVA), Posthoc t-test, and Pearson correlation. The level of significance was set at p˂0.05.

**3. RESULTS**

Table 1 shows that the mean values of MDA and TAC differ significantly across the various groups (p<0.05).The mean values of MDA are significantly lower in control group (2.86 ± 0.61) when compared to Agbo, Alcohol, and Cigarette group (3.80 ± 0.65) and Alcohol and Cigarette group (3.29 ± 0.30) (p<0.05). However, the mean value of TAC is significantly higher in control group (845.26 ± 192.07) when compared to Agbo, Alcohol, and Cigarette group (732.90 ± 130.38) and Alcohol and Cigarette group (709.72 ± 155.98) (p<0.05). The mean value of MDA was significantly higher in Agbo, Alcohol, and Cigarette group (3.80 ± 0.65) when compared to Alcohol and Cigarette group (3.29 ± 0.30) (p<0.05). However, the mean value of TAC did not differ significantly in Agbo, Alcohol, and Cigarette group (732.90 ± 130.38) when compared to Alcohol and Cigarette group (709.72 ± 155.98) (p>0.05).

Table 2 shows the correlation between age and MDA and TAC values in control and test participants. In control participants, there was no significant association between age and MDA (r=-0.089, p=0.619) and TAC (r=0.143, p=0.419) (p>0.05). In participants who consumed Agbo, Alcohol, and Cigarette, there was no significant association between age and MDA (r=-0.124, p=0.483) and TAC (r=-0.115, p=0.517) (p>0.05). In participants who consumed only Alcohol and Cigarette, there was no significant association between age and MDA (r=0.223, p=0.204) and TAC (r=0.073, p=0.680) (p<0.05).

Table 3 shows the correlation between duration of consumption with MDA and TAC values in test participants. In participants who consumed Agbo, Alcohol, and Cigarette, there was no significant association between duration and MDA (r=-0.052, p=0.770) and TAC (r=-0.165, p=0.350) (p>0.05). In participants who consumed only Alcohol and Cigarette, there was no significant association between duration and MDA (r=0.281, p=0.108) and TAC (r=0.037, p=0.837) (p>0.05).

Table .4 shows the correlation between frequency of consumption and MDA an TAC values in test participants. In participants who consumed Agbo, Alcohol, and Cigarette, there was no significant association between duration and MDA (r=-0.220, p=0.211) and TAC (r=-0.058, p=0.743) (p>0.05). In participants who consumed only Alcohol and Cigarette, there was no significant association between duration and MDA (r=0.107, p=0.546) and TAC (r=-0.155, p=0.383) (p>0.05).

**Table 1 The mean values of MDA and TAC in individuals who consume Agbo, Alcohol, and Cigarette and control participants.**

|  |  |  |
| --- | --- | --- |
| Groups | MDA | TAC |
| Control (A) | 2.86 ± 0.61 | 845.26 ± 192.07 |
| Agbo, Alcohol, and Cigarette (B) | 3.80 ± 0.65 | 732.90 ± 130.38 |
| Alcohol and Cigarette (C) | 3.29 ± 0.30 | 709.72 ± 155.98 |
| f-value | 25.353 | 6.854 |
| p-value | <0.001 | 0.002 |
| A vs B | <0.001\* | 0.015\* |
| A vs C | 0.005\* | 0.002\* |
| B vs C | 0.001\* | 1.000 |

*\*significant at p<0.05.*

**Table 2 Correlation between age and MDA and TAC values in control and test participants**

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Parameters | r | p-value |
| Control | Age vs MDA | -0.089 | 0.619 |
| Age vs TAC | 0.143 | 0.419 |
| Agbo, Alcohol, and Cigarette | Age vs MDA | -0.124 | 0.483 |
| Age vs TAC | -0.115 | 0.517 |
| Alcohol and Cigarette | Age vs MDA | 0.223 | 0.206 |
| Age vs TAC | 0.073 | 0.680 |

**Table 3 Correlation between Duration of consumption and MDA and TAC values in test participants**

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Parameters | r | p-value |
| Agbo, Alcohol, and Cigarette | Duration vs MDA | -0.052 | 0.770 |
| Duration vs TAC | -0.165 | 0.350 |
| Alcohol and Cigarette | Duration vs MDA | 0.281 | 0.108 |
| Duration vs TAC | 0.037 | 0.837 |

**Table 4 Correlation between Frequency of consumption and MDA and TAC values in test participants**

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Parameters | r | p-value |
| Agbo, Alcohol, and Cigarette | Frequency vs MDA | -0.220 | 0.211 |
| Frequency vs TAC | -0.058 | 0.743 |
| Alcohol and Cigarette | Frequency vs MDA | 0.107 | 0.546 |
| Frequency vs TAC | -0.155 | 0.383 |

**4. DISCUSSION**

Certain plant ingredients in Agbo contain antioxidants that help neutralize free radicals and reduce oxidative stress (Oreagba *et al*., 2011). The ingredients in these herbal mixtures are considered medicinal as a result of the naturally occurring phytochemicals, with the most abundant class being phenol (Ogbodo *et al*., 2022). This study provides strong evidence that consuming Agbo alongside alcohol and cigarettes increases oxidative stress rather than reducing it. Cigarettes and cannabis have been shown to reduce antioxidants status (Okwara *et al*., 2018; Ezeugwunne *et al*., 2019). Following the statement from Awalu *et al*. (2022), MDA and TAC are considered qualitative indicators for the assessment of oxidant-antioxidant balance in human homeostasis.

The elevated MDA and decrease in TAC levels in the test groups highlight increased lipid peroxidation, a key indicator of oxidative stress. This suggests that the combination of Agbo, alcohol, and cigarettes increases oxidative damage rather than mitigating it. TAC levels, which reflect the body’s ability to neutralize oxidative damage, were significantly lower in test participants, reinforcing the notion that antioxidant defenses were compromised. While Agbo is traditionally believed to have antioxidant properties due to its phytochemical content (e.g., flavonoids and polyphenols), the results suggest that when combined with alcohol and cigarettes, it may contribute to the generation of free radicals rather than counteracting oxidative stress.

Previous studies have highlighted the potential antioxidant effects of polyherbal formulations due to their phytochemical content (Oreagba, 2011). However, the current findings align with studies that suggest polyherbal formulations may have adverse effects when combined with oxidative stress-inducing substances like alcohol and cigarettes (Ali *et al*., 2023). Similar research has shown that alcohol metabolism generates reactive oxygen species (ROS) that increase lipid peroxidation and impair antioxidant defenses (Tsermpini *et al*., 2022), as well as cigarette smoke that contains thousands of free radicals that increases oxidative damage (Aranda-Rivera *et al*., 2022). The present study reinforces the idea that the co-consumption of herbal remedies with these substances may intensify oxidative stress rather than providing protection.

Conclusion

This study reveals that the combined consumption of Agbo, alcohol, and cigarette can reduce antioxidant defenses and enhance the production of free radicals, thereby leading to oxidative stress.

**RECOMMENDATIONS**

Further studies are needed to establish safe consumption guidelines and assess the long-term health implications.

**LIMITATIONS**

Despite the valuable insights provided by this study, certain limitations should be acknowledged. Information on Agbo, alcohol, and cigarette consumption was obtained through self-reported questionnaires, which may be subject to recall bias or underreporting. Different Agbo mixtures may contain varying phytochemical compositions (Folami *et al*., 2024), potentially affecting their interaction with alcohol and cigarettes. Future studies should analyze specific herbal constituents and their biochemical interactions. Additionally, the study provides a snapshot of oxidative stress markers at a single point in time. A longitudinal study would be required to assess long-term effects and potential cumulative damage.

**ETHICAL APPROVAL AND CONSENT**

Ethical clearance to conduct this study was obtained from the Ethics Committee of Faculty of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

**REFERENCES**

Aladejana, E.B. (2023). Biological Properties of Polyherbal Formulations: A Review of their Antimicrobial, Anti-Inflammatory, Antioxidant, and Toxicological Activities. Pharmacognosy Journal, 15(5), 933–963. <https://doi.org/10.5530/pj.2023.15.178>

Ali, J., Rehman, I. U., & Bangash, J. A. (2023). Phytochemicals content and in-vitro antioxidant properties of Azadirachta indica seeds, leaves and twigs prepared from different extraction techniques. International Journal of Engineering Science and Technology, 14(4), 12–20. <https://doi.org/10.4314/ijest.v14i4.2>

Aranda-Rivera, A. K., Cruz-Gregorio, A., Arancibia-Hernández, Y. L., Hernández-Cruz, E. Y., & Pedraza-Chaverri, J. (2022). RONS and Oxidative Stress: An overview of basic concepts. Oxygen, 2(4), 437–478. <https://doi.org/10.3390/oxygen2040030>

Awalu, J. C., Ukibe, N. R., Onyenekwe, C. C., Ahaneku, J. E., Ihim, A. C., Udeh, T., Onah, C. E., Ehiaghe, F. A., Ukibe, G. E., &Ukibe, B. C. (2022). Assessment of oxidative stress and antioxidant status in newly admitted healthy undergraduate students in Nnamdi Azikiwe University, Awka, Nigeria. *Journal of Pharmaceutical Negative Results, 13*(Special Issue 1), 1388-1399. <https://doi.org/10.47750/pnr.2022.13.S01.165>

Benzie IFF, Strain JJ. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. The FRAP Assay. Analytic Biochemistry, 239(1):70-76.

Di Meo, S., & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. Oxidative Medicine and Cellular Longevity, 2020, 1–32. <https://doi.org/10.1155/2020/9829176>

Ezeugwunne, I.P., Okwara, J.E., Onogwu, S., Ogbodo, E.C., Onuora, I.J., Analike, R.A., Amah, A.K., Obi-Ezeani, C.N., Ejiofor, D.C., Nwanegwo, C. (2019). Effect of cannabis smoking on Vitamin C and E levels of male cannabis smokers in Nnewi, Nigeria. *Panacea Journal of Medical Sciences*, 9(1), 15-18. [*http://doi.org/10.18231/j.pjms.2019.005*](http://doi.org/10.18231/j.pjms.2019.005)

Folami, Sulaimon & Abuga, Ibrahim & Balogun, Morufu& Lawal, Abiodun & Lawal, Abubakar &Adetoyi, Gbadebo & Abubakar, Muhammad. (2024). Resident altitude about the usage of herbal medicine in Kwara and Lagos metropolis, Nigeria. Journal of Research in Complementary Medicine. 1. 10.5455/JRCM.20240624082005.

Gutteridge JM and Wilkins S. copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. FEBS Letts., 137:327-330.

Kalyniukova, A., Holuša, J., Musiolek, D., Sedlakova-Kadukova, J., Płotka-Wasylka, J., &Andruch, V. (2021). Application of deep eutectic solvents for separation and determination of bioactive compounds in medicinal plants. *Industrial Crops and Products*, *172*, 114047. <https://doi.org/10.1016/j.indcrop.2021.114047>

Luo, J., Mills, K., Cessie, S. L., Noordam, R., & Van Heemst, D. (2020). Aging, age-related diseases and oxidative stress: What to do next? *Ageing Research Reviews*, *57*, 100982. https://doi.org/10.1016/j.arr.2019.100982

Mussarat, S., M. Adnan, S. Begum, A.A. Alqarawi, S.U. Rehman and E.F. Abd\_Allah. 2021. Diversity of traditionally used polyherbal medicines. Pak. J. Bot., 53(4): DOI: http://dx.doi.org/10.30848/PJB2021-4(15)

 Ogbodo, E.C., Okafor, C.C., Ogah, H.G.O., Ezeugwunne, I.P., Igwebuobi, C.F., Okezie, A.O., Agada, U.N., Amah, A.K., Odumodu, I.O. (2019). Thyroid hormone profiling and enzymatic antioxidant status in diagnosis and management of type-ii-diabetes mellitus: a review of literature. World Journal of Pharmaceutical and Life Sciences, 5(12), 6-21.

Ogbodo, J. O., Agbo, C. P., Echezona, A. C., Ezike, T. C., Emencheta, S. C., Onyia, O. C., Iguh, T. C., &Ihim, S. A. (2022). Therapeutic roles of phenolic antioxidants in herbal medicine. *In* Phenolic Antioxidants in Herbal Medicine (pp. 1-18). ResearchGate. <https://www.researchgate.net/publication/363672273>

Okaiyeto, K., &Oguntibeju, O. O. (2021). African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. *International Journal of Environmental Research and Public Health*, *18*(11), 5988. <https://doi.org/10.3390/ijerph18115988>

Okwara, J.E., Ibe, C.S., Ogbodo, E.C., Analike, R.A., Onyegbule, O.A., Oguaka, V.N., Amah, A.K., Mbaeri, T.U., Meludu, S.C. (2018). The Effects of Cigarette Smoking On the Serum Levels of Some Antioxidants (Vitamin C and E) Amongst Male Smokers in Levels in College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. *International Journal of Scientific Research and Management,* 6(1), 6-11.

Onah, C.E., Onah, C.F., Ehiaghe, F.A., Onyegbule, O.A., Ogbodo, E.C., Ijeomah, A.U., Nnaemeka, W.S., Meludu, S.C., & Dioka, C.E. (2023). Exploring the Therapeutic Potential of *Moringa Oleifera* in Lead Poisoning: An Investigation of its Effects on Blood Lead Levels and Oxidative Stress in Rats. *International Journal of Clinical and Biomedical Research*, 9(2), 1-10.

Oreagba, I. A., Oshikoya, K. A., & Amachree, M. (2011). Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complementary and Alternative Medicine, 11(1). <https://doi.org/10.1186/1472-6882-11-117>

Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R., & Lewandowski, W. (2021). Recent developments in effective antioxidants: the structure and antioxidant properties. Materials, 14(8), 1984. <https://doi.org/10.3390/ma14081984>

Tsermpini, E. E., Ilješ, A. P., &Dolžan, V. (2022). Alcohol-Induced Oxidative Stress and the Role of Antioxidants in alcohol Use Disorder: a systematic review. Antioxidants, 11(7), 1374. <https://doi.org/10.3390/antiox11071374>