EVALUATION OF KIDNEY INJURY MOLECULE-1, CREATININE AND UREA ON POLYHERBAL MEDICINES (AGBO), ALCOHOL AND CIGARETTE CONSUMERS IN NNEWI, NIGERIA.

**Abstract**

 “Agbo”, a traditional polyherbal remedy commonly used in Nigeria, is often relied upon for managing illnesses such as malaria, typhoid, and digestive disorders. These mixtures, made from various plant parts, are believed to offer enhanced healing through the combined effects of multiple herbs. However, growing concerns exist about their potential nephrotoxicity, particularly when taken alongside alcohol and cigarettes. These substances, individually known to impair kidney function, may act synergistically to worsen renal stress. This study assesses the impact of polyherbal medicines (Agbo), alcohol, and cigarette use on kidney function by measuring creatinine, urea, and Kidney Injury Molecule-1 (KIM-1) levels. It compares consumers and non-consumers and examines how age, frequency, and duration of use relate to these biomarkers. This is a comparative cross-sectional study involving 102 participants divided into three equal groups comprising 34 individuals each: non-consumers (control), Agbo-alcohol-cigarette consumers (AAC), and alcohol-cigarette consumers (AC). Blood samples were collected and analyzed for serum kidney injury molecule-1 (KIM-1), creatinine, urea, and using ELISA, Jaffe-slot, and Berthelot methods, respectively. Data on age, duration, and frequency of substance use were collected via questionnaires. Statistical analysis, including ANOVA and Pearson correlation, were performed using SPSS version 26, with significance set at p < 0.05 to evaluate differences and relationships across the groups. Results showed that serum levels of creatinine, urea, and KIM-1 differed significantly among the three groups (p < 0.05). Post-hoc analysis revealed that the mean levels of KIM-1, creatinine and urea were significantly higher in ACC group when compared with the control group (p < 0.05). However, only KIM-1 level was significantly higher in AC group when compared with control group (p < 0.05). Creatinine and urea levels did not differ significantly in AC group when compared with control group (p>0.05). Duration of intake and age did not correlate significantly with the KIM-1, creatinine and urea in various groups (p > 0.05). In conclusion, these findings indicate that the combined intake of Agbo, alcohol, or cigarettes may exacerbate kidney dysfunction.

**Keyword**: Polyherbal medicine; Agbo; Kidney function; Nephrotoxicity; Creatinine; Urea; Kidney Injury Molecule-1 (KIM-1); Alcohol consumption; Cigarette smoking; Traditional medicine; Oxidative stress; Acute kidney injury (AKI); Chronic kidney disease (CKD); Herbal toxicity.

**Introduction**

Polyherbal medicines (Agbo) involve the combination of multiple medicinal herbs, these formulations are designed to maximize therapeutic efficacy through synergistic effects of the different herbal components, and they are increasingly used in the treatment of chronic diseases, including diabetes, cardiovascular disorders, and inflammatory conditions (Umaru *et al*., 2025). Several herbs commonly used in this Polyherbal medicines (Agbo) have been documented to have nephrotoxic properties. For instance, a study by Kumar *et al*. (2021) highlighted that herbs such as *Aristolochia spp*. and *Huperzia serrata*, often included in traditional mixtures, are associated with renal toxicity. The study demonstrated that chronic exposure to these herbs led to a significant increase in serum creatinine and urea levels in animal models, indicating impaired renal function (Kumar *et al*., 2021). Polyherbal medicine (Agbo) may interact with prescription medications, exacerbating kidney damage or impairing drug metabolism (McGraw and Sharma, 2020). Some herbal products may contain heavy metals, which can as well lead to cumulative renal toxicity and impaired kidney function (Ali *et al*., 2023).

However, several studies have highlighted the nephroprotective effects of these Polyherbals. A study by Kumar *et al*. (2021) demonstrated that a polyherbal formulation containing *Tribulus terrestris*, *Phyllanthus niruri*, and *Vigna mungo* significantly reduced renal damage induced by gentamicin in rats. The formulation was shown to mitigate oxidative stress and inflammation, thereby protecting renal tissue from injury. These findings suggest that specific combinations of herbs can enhance renal protection and improve outcomes in patients at risk for nephrotoxicity (Kumar *et al*., 2021).

Alcohol consumption has been identified as a potential risk factor for kidney impairment. Studies have shown that chronic alcohol intake disrupts renal function, leading to reduced glomerular filtration rate (GFR) and increased oxidative stress. Xu *et al*. (2021) conducted a Mendelian randomization study and found a significant association between heavy alcohol consumption and a decline in kidney function. This is supported by research from Das *et al*. (2017), which highlights that ethanol-induced oxidative stress plays a major role in kidney damage by increasing free radical production and reducing antioxidant defense mechanisms. Hall *et al*. (2018) reported that individuals with prolonged alcohol consumption had an increased likelihood of developing hypertension-induced renal damage, contributing to progressive kidney dysfunction.

Hall *et al*. (2018) found that smokers were more likely to develop proteinuria compared to non-smokers, suggesting a direct link between cigarette exposure and nephron damage. Yacoub *et al*. (2017) reviewed the mechanisms by which cigarette smoking induces renal injury, emphasizing that nicotine and other toxic compounds in cigarettes lead to vasoconstriction, increased blood pressure, and glomerular damage

The kidneys play a crucial role in maintaining homeostasis, regulating fluid balance, electrolytes, and waste excretion. Chronic kidney disease (CKD) has become a significant public health issue worldwide, and there is growing interest in the potential of Polyherbal medicines (Agbo) to support renal health. Elevated creatinine levels indicate impaired glomerular filtration rate (GFR), while high urea levels suggest decreased renal clearance and compromised kidney function (StatPearls, 2023). In addition to these traditional markers, Kidney Injury Molecule-1 (KIM-1) has emerged as a sensitive biomarker for early kidney injury. KIM-1 expression significantly increases in response to tubular damage, providing early detection of acute kidney injury (AKI) even before changes in serum creatinine (Bansal & Nigoskar, 2023). While Polyherbal medicines (Agbo) are often used for their potential therapeutic effects, there is growing concern about their nephrotoxic side effects. Therefore, this study evaluates the impact of polyherbal medicines (Agbo), alcohol, and cigarette use on kidney function by measuring creatinine, urea, and Kidney Injury Molecule-1 (KIM-1) levels.

**Materials and method**

**Study Design and Population**

This study employed a comparative cross-sectional design, categorizing participants into three distinct groups based on their consumption habits. These involve 102 consecutive consenting adults, divided into three age-matched groups of 34 participants each: Group A (Control Group), consisting of individuals who did not consume Agbo, alcohol, or cigarettes; Group B (AAC Group), consisting of individuals who regularly consumed Agbo (polyherbal medicines), alcohol, and cigarettes; and Group C (AC Group), consisting of individuals who consumed alcohol and cigarettes but did not take Agbo. The study was approved by the Ethics Committee of the Faculty of Medical Laboratory Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

The inclusion criteria for the study were individuals aged 25 to 60 who resided in Nnewi and consumed Agbo, alcohol, or cigarettes, while the exclusion criteria included individuals residing outside Nnewi, those who declined participation, and those under 25 or over 60 years old; additionally, data were collected from a control group of individuals who did not consume Agbo, alcohol, or cigarettes.

**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**

Data was collected from study participants through a questionnaire, gathering demographic information such as age, gender, ethnicity, medical history, and duration of intake of these substances. Blood samples were collected, and serum was extracted and stored for one month before analysis. Laboratory methods included three assays: the Enzyme-linked Immunosorbent Assay (ELISA) for Kidney Injury Molecule-1 (KIM-1) using the method as described by Zhu *et al*. (2025); the Jaffe reaction for creatinine using the method as described by Jaffe, (1886), and the Berthelot reaction for urea using the method as described by Berthelot, (1859).

**Statistical Analysis**

Data analysis was performed using SPSS version 26. Descriptive statistics (mean ± standard deviation) were used to summarize the data. A one-way analysis of variance (ANOVA) was conducted to compare biomarker levels across groups, followed by Bonferroni post-hoc tests for pairwise comparisons. Pearson correlation analysis was used to assess the relationships between biomarkers and demographic factors. Statistical significance was set at p < 0.05.

**Results**

Table 1 presents the mean and standard deviation (SD) of serum creatinine, urea, and KIM-1 levels across three study groups: Control, AAC (Agbo-Alcohol-Cigarette users), and AC (Alcohol-Cigarette users). Statistical analysis revealed significant differences in the mean levels of KIM-1, creatinine and urea among the three groups (p < 0.05), with the AAC group having significantly higher levels of all biomarkers compared to the control group. The AC group showed a significantly higher KIM-1 level compared to the control, but no significant difference was observed in creatinine or urea between the AC and control groups. Furthermore, the AAC group had significantly higher creatinine levels than the AC group, but no significant difference was found in urea or KIM-1 levels (p>0.05).

Table 2 indicates that there were no significant correlations between age and the various parameters in control, AAC and AC groups (p>0.05).

Table 3 shows that the duration of Agbo, alcohol, and cigarette consumption had no significant correlation with any biomarkers in AAC and AC groups (p>0.05).

Table 4 reveals no significant correlation between the frequency of intake with KIM-1, creatinine or urea in ACC and AC group (p>0.05).

Table 1: The mean value of Creatinine, Urea and KIM-1 of the participants (Mean ± SD)

|  |  |  |  |
| --- | --- | --- | --- |
| GROUPS | CREATININE  | UREA | KIM-1 |
| CONTROL (A) | 80.91±18.81 mmol/L | 3.03±0.44 mmol/L | 9.45±2.75 ng/L |
| AAC (B) | 95.88±15.44 mmol/L | 3.47±0.93 mmol/L | 14.3218±4.64 ng/L |
| AC (C) | 84.71±15.53 mmol/L | 3.11±0.62 mmol/L | 13.28±2.95 ng/L |
| F-VALUE | 7.42 | 3.82 | 17.73 |
| P-VALUE | 0.001 | 0.025 | <0.001 |
| A VS B | 0.001 | 0.034 | <0.001 |
| A VS C | 1.000 | 1.000 | <0.001 |
| B VS C | 0.020 | 0.103 | 0.683 |

Statistically significant at p<0.05

AAC = Agbo, alcohol and cigarette

AC = Alcohol and cigarette

Table 2: Correlation of Age with Biochemical Biomarkers

|  |  |  |  |
| --- | --- | --- | --- |
| GROUP | PARAMETERS | R | P-VALUE |
| CONTROL | AGE VS CREATININE  | 0.058 | 0.746 |
|  | AGE VS UREA | -0.196 | 0.266 |
|  | AGE VS KIM-1 | -0.006 | 0.974 |
| AAC | AGE VS CREATININE  | -0.034 | 0.849 |
|  | AGE VS UREA | -0.120 | 0.500 |
|  | AGE VS KIM-1 | -0.062 | 0.726 |
| AC | AGE VS CREATININE  | -0.189 | 0.223 |
|  | AGE VS UREA | -0.079 | 0.658 |
|  | AGE VS KIM-1 | 0.297 | 0.088 |

Statistically significant at p<0.05

Table 3: Correlation of Duration with Biochemical Biomarkers

|  |  |  |  |
| --- | --- | --- | --- |
| GROUP | PARAMETERS | R | P-VALUE |
| AAC | DURATION VS CREATININE  | -0.106 | 0.549 |
|  | DURATION VS UREA | 0.004 | 0.983 |
|  | DURATION VS KIM-1 | -0.052 | 0.770 |
| AC | DURATION VS CREATININE  | -0.305 | 0.080 |
|  | DURATION VS UREA | -0.118 | 0.507 |
|  | DURATION VS KIM-1 | 0.221 | 0.208 |

Statistically significant at p<0.05

Table 4: Correlation of Frequency with Biochemical Biomarkers

|  |  |  |  |
| --- | --- | --- | --- |
| GROUP | PARAMETERS | R | P-VALUE |
| AAC | FREQUENCY VS CREATININE  | -0.017 | 0.925 |
|  | FREQUENCY VS UREA | -0.179 | 0.227 |
|  | FREQUENCY VS KIM-1 | -0.099 | 0.578 |
| AC | FREQUENCY VS CREATININE  | 0.141 | 0.247 |
|  | FREQUENCY VS UREA | 0.083 | 0.640 |
|  | FREQUENCY VS KIM-1 | -0.039 | 0.828 |

Statistically significant at p<0.05

**Discussion**

This study investigated the impact of polyherbal medicine (Agbo), alcohol, and cigarette consumption on kidney function by analyzing key biomarkers—Creatinine, Urea, and Kidney Injury Molecule-1 (KIM-1). Herbal medicines are widely perceived as natural and safe, but many contain compounds that can be nephrotoxic at certain doses. The significant increase in KIM-1, creatinine and urea levels in the AAC group suggests that polyherbal medicines, when combined with alcohol and cigarettes, may accelerate kidney damage. This aligns with findings from Ammari *et al*. (2024), who reported that higher doses of a polyherbal extract formulation led to histological changes indicative of kidney injury. Additionally, a study by Enemali *et al*. (2023) demonstrated that the ethanol extract of *Phyllanthus urinaria* affected liver and kidney function parameters in paracetamol-administered albino rats, highlighting the potential impact of herbal extracts on renal health. Other studies by Analike *et al*. (2018), Ezeugwunne *et al*. (2018) and Ogbodo *et al*. (2017) also showed that the ethanol extract of *Sida corymbosa* affected both the liver and kidney function indices in alloxan-induced diabetic albino wistar rats which aligns with the current findings.

The combined effects of alcohol, and cigarettes only also increased the level of KIM-1 even though it did not alter the levels of creatinine and urea. This shows that the combination of alcohol and cigarettes can induce kidney injury. However, the effect of the combination of the three substances seems to be more detrimental to the kidney. According to a study by Ammari *et al*. (2024), chronic exposure to multiple substances can lead to significant changes in kidney biomarkers and histological alterations. Similarly, Enemali *et al*. (2023) found that herbal extracts, when consumed with other substances like paracetamol, affected kidney function markers. These findings highlight the importance of regulating polyherbal medicine use and discouraging combined substance abuse to reduce the risk of kidney dysfunction.

This study revealed that polyherbal medicine (Agbo), alcohol, and cigarette use may significantly impact negatively on kidney functions. The elevated KIM-1 and creatinine levels in this group suggests that their intake regardless of duration and frequency may contribute to renal injury.

**Recommendations**

Regular kidney function screening is advised for individuals who frequently consume these substances to enable early detection and intervention.

**Ethical Approval and Consent**

We hereby declare that this study was examined and approved by the Ethics Committee of the Faculty of Medical Laboratory Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained from all individuals that participated in the study.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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