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Evaluation of the Combined Extract of *Rauwolfia vomitoria* and *Aframomum melegueta* on the Kidney and Liver of Adult Male Wistar Rats

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Abstract

Phytomedicine, also known as herbal medicine, has seen a great upsurge globally. It refers to the use of plants, herbs, and mixtures of well-known plant metabolites to cure and mitigate human diseases. They are often used because they are natural and therefore assumed to be safer than allopathic medicines. In recent times, however, there has been a growing concern about their safety. This study aimed to evaluate the combined effect of methanolic extract of *Rauwolfia vomitoria* and *Aframomum melegueta* on renal and liver morphology of experimental animals with respect to the expression of Interleukin 10 (IL-10) and nuclear factor erythroid 2-related factor 2 (NRF2). Twenty adults male Wistar rats weighing 180-230g were grouped into four consisting of a control and three test groups. While the control rats were administered with distilled water only, the rats in groups II and III were singly administered with methanolic extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* at standard doses of 250mg/kg/BW respectively while rats in group IV were co-administered with the methanolic extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* at 250mg/kg/BW respectively. At the end of the experiment, the rats were euthanized, and the kidneys and livers were analysed for histological and mRNA analysis. Histopathological findings revealed no cytopathic lesions on the renal and liver morphology across all groups while mRNA expression studies indicated the anti-inflammatory and antioxidant effects of the plants by upregulating the expression of IL-10 and Nrf-2 in the treated groups. The administration of the leaf extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* at a dosage of 250mg/kg/BW has no cytopathic effects on the kidney and liver morphology of wistar rats with the co-administrative extract treatment better upregulating the expression of IL-10 and NRF-2 than single administration in the treated rats thus indicating its antioxidative potential and safety of usage and further validating their use in traditional medical practice.

Keywords: Phytomedicine, *Rauwolfia vomitoria*, *Aframomum melegueta*, Interleukin-10, Nuclear factor erythroid 2-related factor 2 (NRF-2).

Introduction

Phytomedicine is the use of plants and herbs and mixtures of well-known plant metabolites to cure and mitigate human diseases (Imaga, 2010, Mothibe and Sibanda 2019; Rashid *et al.*, 2021; Coopoosamy *et al.,* 2023). They are natural and assumed to be safer than allopathic medicines which encourages high frequent usage (Yunes *et al.,* 2012, Mensah *et al.,* 2019). *Rauwolfia vomitoria Afzel* (Apocyanaceae) is one of the medicinal plants that have served all through the ages in the treatment and preservation of human health (Iroghama *et al.,* 2018). *Rauwolfia vomitoria is* called serpent wood, serpent snake root and swizzle stick, as well as "asofeyeje" in Yoruba, "ira" in Igbo, "akata" in Bini and "Mmoneba and utoenyin" in Efik, and "wadda" in Hausa and it is commonly found in southern Nigeria, West Africa (Eluwa *et al.,* 2010; Peter and Ekpene, 2021). It belongs to the Apocyanaceae family, and it is described as a small tree or rainforest large shrub that grows to

an 8m height (about 26ft high). Its branches grow in whorls, and the leaves grow from small nodes in groups of three. The leaf blades are broadly lanceolate or elliptical, usually, acuminating. Its flowers are small and fragrant and are followed by globular red fruit. All parts of the plant except the mature wood, contain latex. The parts that are commonly used for herbal remedies are roots, bark, root bark, leaves, and stem bark (Eluwa *et al.,* 2010). The extract from *R. vomitoria* was extracted first in 1952 by Swiss Chemists, they were the first to discover this plant as a natural neuroleptic (Amole and Onabanjo, 1999, Asoro *et al.,* 2018). *R. vomitoria* is used as malaria reducing agent and snakebite antidote; the roots can be powdered to treat diarrhoea and dysentery, while the latex from the leaves is also used in treating parasitic skin disease (Eteng *et al.,* 2009). Reports by Eteng *et al*. (2009) also documented that Ghanaian and Nigerian traditional healers use the bark of *R. vomitoria* as an emetic and purgative in the management of gastrointestinal tract discomfort, jaundice, and infantile convulsion.

Aframomum melegueta is popularly known as grains of paradise, Guinea pepper, alligator pepper as well as "atare" in Yoruba, "chitta" in Hausa, "ose oji" in Igbo. It is an herbaceous perennial plant in the ginger family (Zingiberaceae) (Grains of Paradise). It is a spice with a similar composition as Ginger that belongs to the same Zingiberaceae family. Taxonomically, it is classified as follows: Plantae (kingdom), Tracheophyta (phylum), Liliopsida (class), Zingiberales (order), Zingiberaceae (family), *Aframomum* (genus) and *Aframomum melegueta* (species). It is commonly found in abundance in Western African countries- Nigeria, Ghana, Liberia, Ivory Coast, and Togo. Specifically, it is native to the swampy habitat along the coast of Nigeria (Osuntokun, 2020a). It is often described as a plant that has trumpet-shaped purple flowers that develop into 5 to 7-cm-long pods. These pods contain small, numerous edible reddish-brown seeds, having the pungent smell of ginger and cayenne pepper. The stem bark is short and covered with scars of fallen leaves. The leaves are about 30 cm long, 12 cm wide, with a well-structured vascular system (Osuntokun, 2020b).

The macerated seeds of *Aframomum melegueta* have been reported to relieve inflammation and pain following its application to swollen parts of the body (Umukoro and Aladeokin, 2011). Experimental studies have also established that the crude extract of *A. melegueta* seeds exhibited anti-inflammatory potential by inhibiting prostaglandin synthesis as well as analgesic activity by inhibiting both acute and chronic inflammatory responses, which provides the basis for its use in traditional medicine for acute and chronic inflammatory disorders (Umukoro and Aladeokin, 2011). The aqueous extract of *A. melegueta* has been reported to confer hepato-protective properties irrespective of its dosage (Chiejina and Ukeh, 2012). Also, it has bacteriostatic and bactericidal activities which makes it useful in the treatment of infectious diseases- urinary tract infection caused by *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus saprophyticus, Proteus mirabilis, methicillin-resistant Staphylococcus aureus, Salmonella spp, and Shigella spp* (Alo *et al.,* 2012). The single use of *Rauwolfia vomitoria* in phytomedicine has been reported not to have a toxic effect on the renal morphology of experimental animals as the integrity of the kidneys is maintained as well as its histoarchitecture (Ibrahim and Onanuga, 2014; Oyewole and Massaquoi 2019). Reports by Obike *et al*. (2014) also indicated that there was no significant effect on the body or organ weight of rats that were treated with *A. melegueta*. Furthermore, histoarchitectural studies showed no alteration in the kidneys of rats administered with the plant at an elevated or reduced concentration. Interleukin-10 (IL-10) is an anti-inflammatory pleomorphic cytokine with different phenotypic effects and its principal actions are anti-inflammatory, inhibitory, or self-regulating (Steen *et al.,* 2020). It is a product of T helper 2 cells produced by activated immune cells (O'Garra *et al.,* 2008). Conversely, Nuclear factor erythroid 2-related factor 2 (NRF-2) is a transcription factor activated by multiple stimuli (Bia *et al.,* 2015). Sahin *et al.* (2012) reported that NRF-2 plays an important antioxidant role in protecting the cells against oxidative stress. Reports regarding the combined effects of extracts from both *plants* on the morphology of

the kidney and liver are scanty in existing literature. Hence, this present study was carried out to investigate the combinatorial sub-acute effects of methanolic leaf extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* and to ascertain its toxicity on the renal and liver morphology of adult male Wistar rats. In the same vein, the expression of the IL-10 and NRF-2 markers on the kidney and liver was determined to measure the potential protective effect of the combined extracts.

Materials and Methods

Study design and Experimental animals.

This research was a cross-sectional experimental study carried out between June-August 2023 on adult male Wistar rats at the histopathology unit of the Department of Medical Laboratory Sciences, University of Medical Sciences, Ondo City, Ondo State (UNIMED) located in the Southwest geopolitical zone of Nigeria. A total of 20 adult male Wistar rats obtained from the vivarium of UNIMED were used in this study.

Research ethical approval.

The ethical approval for this study was sought and approved by the Ministry of Agriculture, Akure, Ondo State, which is consistent with those set down by the National Institute of Health (NIH) in the *"Guide to the Care and Use of Animals in Research and Teaching"* (National Academy of Science [NAS], 2011). *The ethical approval is with numbers* MNR/V.384/36 and MNR/V.384/35 respectively.

Plant collection, extraction, and preparation.

Fresh leaves of *Rauwolfia vomitoria* and *Aframomum melegueta* were harvested from a farm in Laje Village Ondo town, Ondo State. The leaves were identified and authenticated by a taxonomist from the Department of Plant Biology and Biotechnology, UNIMED Ondo and assigned herbarium numbers, UNIMED P.B.T.H No. 031 and 030 respectively. The fresh leaves of the plants were washed in running tap water to remove dirt, shade dried, and grinded into a fine powder using a laboratory pulveriser. The powdered leaves were soaked in 95% methanol, allowed to stay for 24 hours, and extracted using a Soxhlet evaporator. Afterwards, the extracts were evaporated using a rotary evaporator. The extracts obtained were allowed to dry in an oven at 45°C and stored in the refrigerator until required for administration (Ekong *et al*., 2015). The plant extracts were administered to the rats at standard doses of 250mg of this extract /kg body weight with the co-administrative treatment of *R. vomitoria* and *A. melegueta* administered at 250mg/kg/bw respectively.

Experimental design

Twenty adults male Wistar rats obtained from the animal holding of UNIMED were acclimatized for two weeks before commencing the experiment. The rats were randomly divided into four groups consisting of five rats each. The first group consisted of the unexposed negative control rats that were allowed access to water and rat chow *ad libitum* while the second group was orally administered with the extract of *R. vomitoria* of standard dosage 250mg/kg/BW. The third group was orally administered with extract of *A. melegueta* at the standard dosage of 250mg/kg/BW while the last group was co-administered with the combined extract of *R. vomitoria* and *A. melegueta* at a standard dosage of 250mg/kg/BW respectively. The duration of the administration was 28 days across all the test groups.

Specimen collection

Upon completion of the experiment, the rats were euthanized using cervical dislocation and the kidneys and livers were carefully excised and immediately transferred into 10% neutral buffered formalin for fixation and were processed using the tissue paraffin embedding technique (Adeniyi *et al.,* 2023). The samples for mRNA expression of IL-10 and NRF-2 were carefully rinsed in Phosphate Buffered Saline (PBS) and analyzed appropriately.

mRNA analysis

For the mRNA analysis, RNA was harvested from the kidney and liver tissues and the expression level was determined by PCR as described by . Briefly, RNA was purified from 100-200 mg from the tissues using TRIZOL reagent (Inqaba Biotech West Africa Ltd) as described by the manufacturer (InvitrogenTM, Denmark). The concentration and purity of isolated RNA was determined at OD260nm and OD260/OD280nm absorption respectively.

RNA was used for downstream application if the ratio falls between 1.8 and 2.1. The extracted RNA (2 µl) was used for the reverse transcription reaction to synthesize cDNA using ProtoScript II First Strand cDNA Synthesis kit (Biolabs, New England) in a 3-step reaction condition: 65°C for 5 minutes, 42°C for 1 hour and 80°C for 5 minutes. Polymerase Chain (PCR) and amplification for gene expression was done using Luna Mastermix kit (Biolabs, New England) and Taqman kit probes from TibM01bio (Berlin, Germany) in a thermocycler. Gel imaging was performed on an electrophoresis gel imager, using β-actin as the reference gene. Primers to cDNA were purchased from Inqaba biotech (Hatfield, South Africa). The primers used for PCR are listed with their corresponding sequence.

The primers for IL-10 are.
Forward primer- 5'-TTGAACCACCCG GCATCTAC-3'
Reverse primer- 5'-CCAAGGAGTTGC TCCCGTTA-3'

The primers for NRF-2 are.
Forward primer- 5' -GGGGAACAGA ACAGGAAACA-3'
Reverse primer- 5' -CCGTAATGCACG GCTAAGTT-3'

The primers for β-actin are.
Forward primer- 5' -CCCGCGAGTACA ACCTTCT-3'
Reverse primer- 5' -CGTCATCCATG GCGAACT-3'

Gel electrophoresis of PCR products was used to analyze the reaction quality and yield of DNA products.

Statistical analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p \leq 0.05$.

Result

Histological Finding

The histological findings indicated that the kidney and liver sections of the rats from the unexposed control group were devoid of pathological lesions. Findings from the treated groups (groups 2 to 4) indicated that the extracts of *R. vomitoria* and *A. melegueta either singly or combined* does not induce any cytopathic effect on the kidney and liver of the treated rats. The kidney of unexposed rats showed normal glomeruli, and renal tubules (Figure 1A). The liver of unexposed rats showed normal morphology of the hepatocytes, the sinusoids and Kupffer cells appeared normal devoid of pathological lesions (Figure 1B). Similarly, the kidney of exposed rats administered with the methanolic extract of *R. vomitoria* shows normal glomeruli and normal renal tubule (Figure 2A). The liver of rats administered with methanolic leaf extract *R. vomitoria* showed the morphology of hepatocytes appearing normal, the sinusoids and the Kupffer cell appeared normal without inflammation (Figure 2B). The kidney of exposed Wistar rats administered with *A. melegueta* methanolic leaf extract showed glomeruli and renal tubules which appeared normal (Figure 2C). Liver of rats administered with methanolic leaf extract *A. melegueta* showed the morphology of hepatocytes which appeared normal, and sinusoids appeared devoid of inflammatory cells (Figure 2D). Furthermore, the kidney section of rat co-administered with the leaf extracts of *A. melegueta* and *R. vomitoria* at standard doses of 250mg/kg respectively showed normal glomeruli and normal renal tubules (Figure 3A) while the liver histology also appeared normal typified by normal appearing hepatocytes with sinusoids devoid of inflammation or congestion (Figure 3B).

mRNA analysis

The mRNA studies indicated that the methanolic extracts of *R. vomitoria* and *A. melegueta* elevated the expression of IL-10 levels in the treated rats relative to the control while the co-administrative treatment with both plant extracts significantly elevated IL-10 expression ($p<0.05$). However, comparative analysis with each extract was not significant ($p>0.05$). (Figure 4). The mRNA expression levels of Nrf-2

are shown in Figure 5. A significantly elevated expression was observed in the co-administrative treatment relative to other groups while single administration of individual plants significantly elevated the expression of Nrf-2 when compared to the control. Additionally, *A. melegueta* significantly increased the expression of Nrf-2 compared to *R. vomitoria* further suggesting a higher antioxidative properties ($p<0.05$).

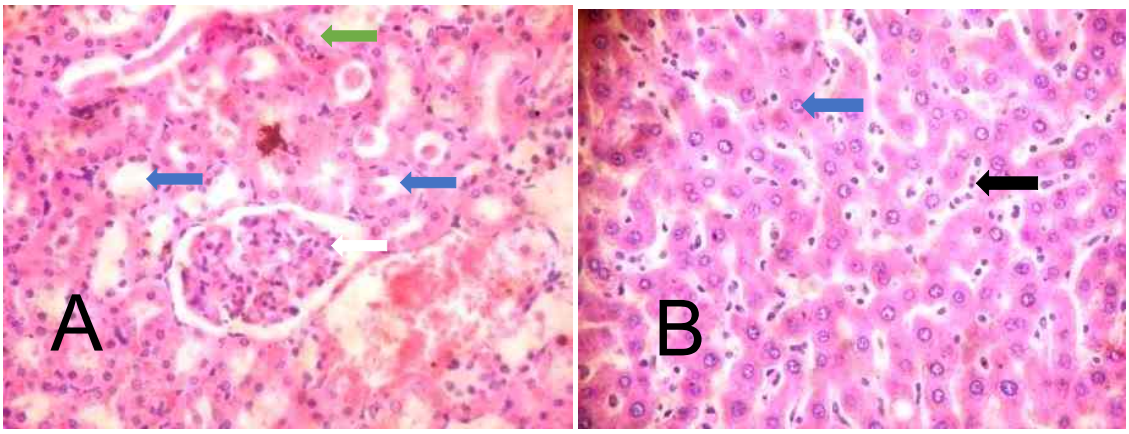

Figure 1. H & E-stained section. a. control kidney section of unexposed rats shows normal glomerulus (white arrow), renal tubules with some showing eosinophilic cast within their luminar spaces (blue arrow), and interstitial spaces devoid of congestion or inflammation (green arrow). **b**. liver section of unexposed control rats showing normal morphology of the hepatocytes (blue arrow), the sinusoids appear normal (black arrow) (x400).

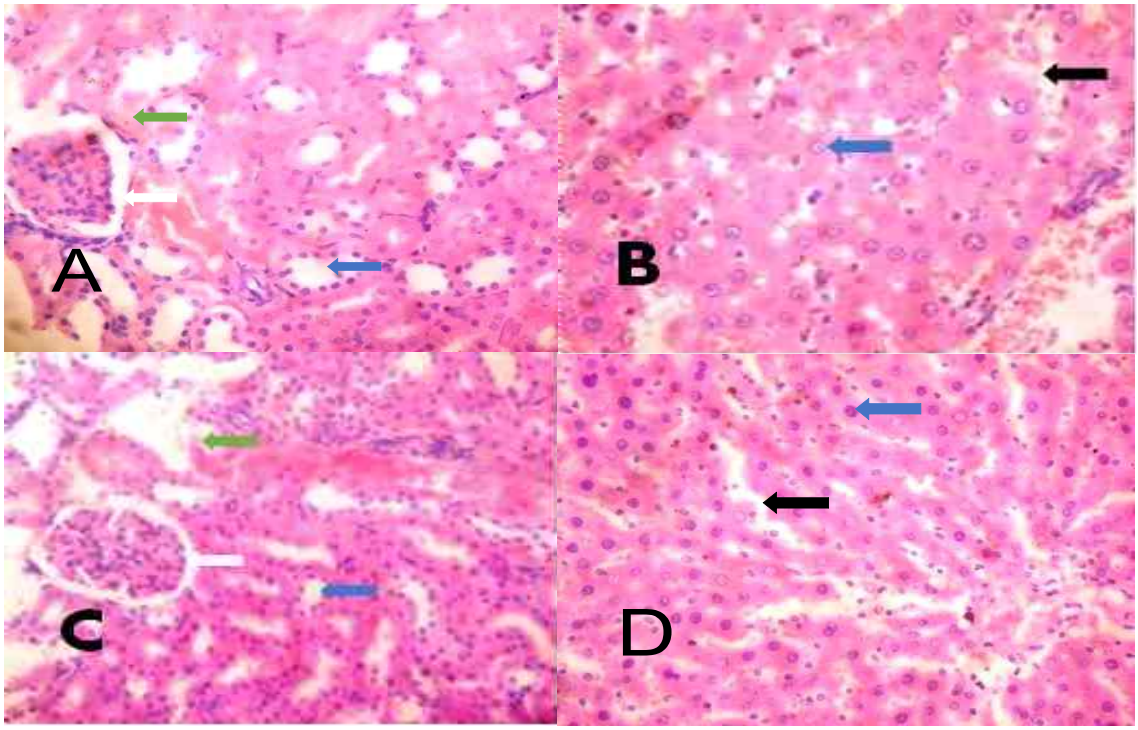

Figure 2 H & E-stained section. **a.** kidney section of rat administered with methanolic leaf extract *R. vomitoria* (250mg/kg/bw) shows normal glomeruli (white arrow), renal tubule (blue arrow). **b**. liver section of rat administered with methanolic leaf extract *R. vomitoria* showing normal hepatocytes (blue arrow), the sinusoids appeared normal and devoid of inflammation of congestion (black arrow). **c**. Rats administered with methanolic leaf extract *A. melegueta* (250mg/kg/bw) showing normal glomeruli (white arrow) and renal tubules (blue arrow). **d.** liver of rat administered with methanolic leaf extract *A. melegueta* showing a normal morphology of hepatocytes appear normal (white arrow), the sinusoids appear devoid of inflammation or congestion (black arrow) (x400).

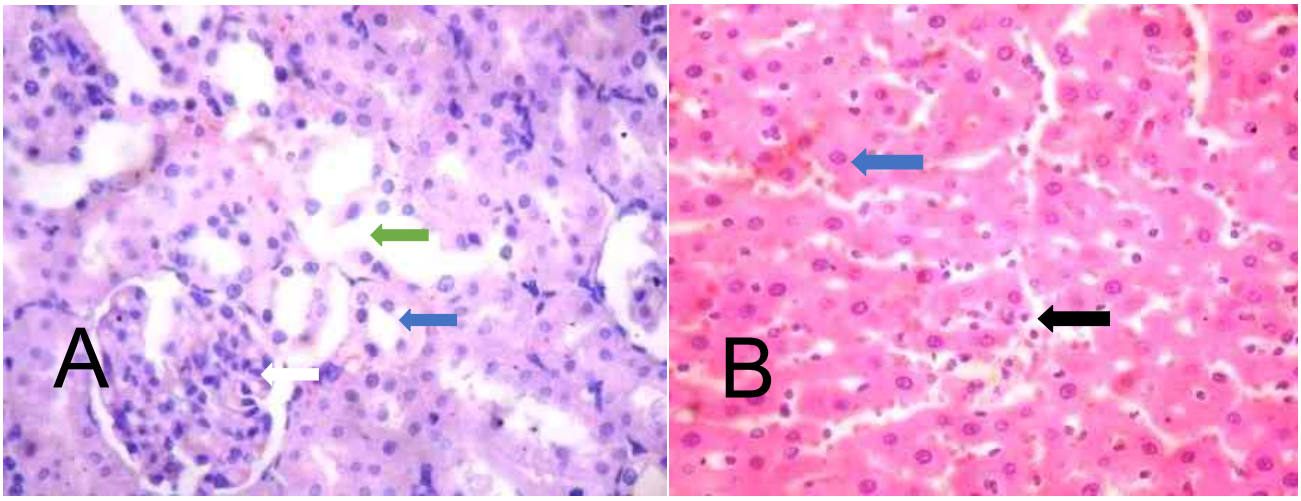

Figure 3. H & E-stained section. **a.** kidney section of rat co-administered with methanolic leaf extracts of *A melegueta* and *R vomitoria at standard doses of 250mg/kg/bw respectively show* normal glomeruli (white arrow) and normal renal tubules (blue arrow), the interstitial space appears normal (green arrow). **b**. liver section of rat co-administered with methanolic leaf extract of *A. melegueta* and *R. vomitoria at standard doses of* 250mg/kg respectively showing a normal morphology of the hepatocytes (white arrow), the sinusoids appear normal without inflammation (black arrow) (x400).

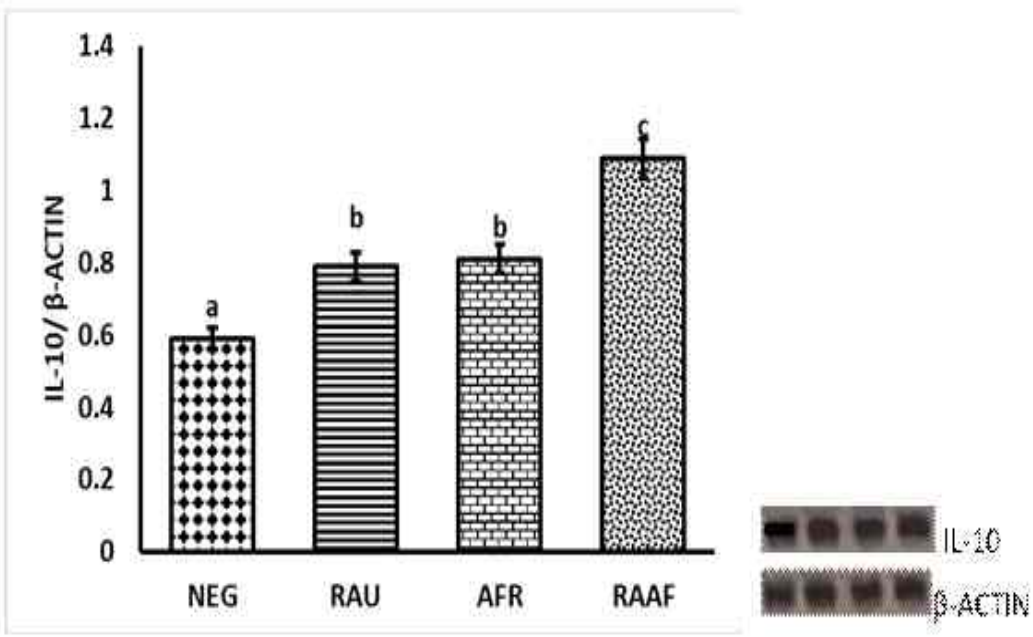

Figure 4: mRNA expression of IL-10 across the groups analysed using quantitative RT-PCR.
Key
NEG: Negative, unexposed control group, **RAU**: Rats administered with *R. vomitoria*, **AFR**: Rats administered with *A. melegueta*, **RAAF**: Co-administrative treatment with *R. vomitoria* and *A. melegueta*.

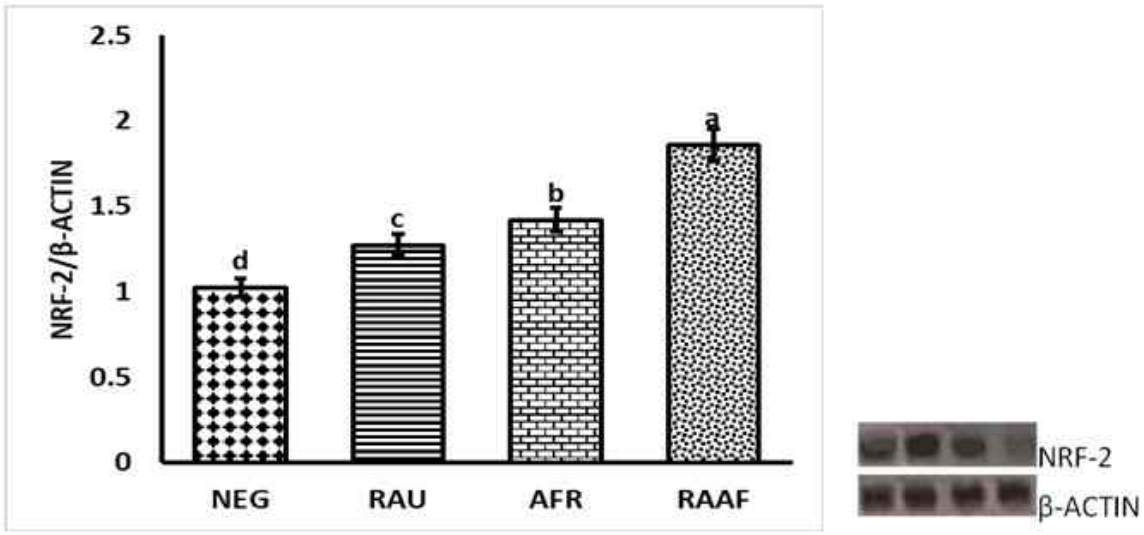

Figure 5: mRNA expression of Nrf-2 across the groups analysed using quantitative RT-PCR.
Key
NEG: Negative, unexposed control group, **RAU**: Rats administered with *R. vomitoria*, **AFR**: Rats administered with *A. melegueta*, **RAAF**: Co-administrative treatment with *R vomitoria* and *A. melegueta*.

Discussion

Phytomedicine has been in existence and demonstrated to be an effective remedy for decades for a variety of ailments, however, the abuse of these medicinal plants can be deleterious to human health if mismanaged (Shing *et al.,* 2019). In this study, we evaluated the safety of *Rauwolfia vomitoria* and *Aframomum melegueta* which are medicinal plants used in the local management of various ailments by traditional healers in Ondo town. We also evaluated the antioxidative potential of the plants by estimating the expression of IL-10 and Nrf-2 in the experimental animals. Generally, we observed that administration of these plants either singly or co-administrative treatment at the studied dosage is safe and not toxic as no histoarchitectural alteration was observed in the studied organs further justifying its usage by traditional healers. We also observed an upregulation in the expression of IL-10 and Nrf-2 in the treatment group with the co-administrative treatment having a significantly elevated level in the studied markers. Studies have documented the anti-inflammatory as well as analgesic activity potential of *Aframomum melegueta* (Umukoro and Aladeokin, 2011). Furthermore, reports on the hepatoprotective hepato-protective properties irrespective of its dosage have been documented (Chiejina and Ukeh, 2012). In this study, the histoarchitectural evaluation of the kidney of the unexposed control rat administered with distilled water and rat chow appeared normal with the glomeruli and renal tubules maintaining their histology which agrees with the reports of Ibrahim and Onanuga (2014). Additionally, treatments with *Rawuolfia vomitoria* showed no alteration in the histoarchitecture, as no pathological lesion was seen in the kidney which aligns with the reports of Oyewole and Massaquoi (2019) that documented that the oral administration of *R. vomitoria* does not induce any cytopathic lesions in the kidney. The rats administered with the methanolic extracts of *Aframomum melegueta* showed normal glomeruli and renal tubules devoid of any cytopathic lesions further suggesting the safety of the plant at the studied dosage. Reports by Obike *et al.* (2014) documented that *Aframomum melegueta* has no damaging effect on the kidney architecture of rats which is in tandem with the findings in this study. Furthermore, the co-administration of the methanolic extracts of *A. melegueta* and *R. vomitoria* maintained the histoarchitecture of the kidneys which is in concord with the reports of Oyewole and Massaquoi, (2019). Histoarchitectural evaluation of the liver after

administration of the extracts indicated no toxicity and cytopathic effect on the liver as the hepatocyte, sinusoid and portal vein appeared normal devoid of any pathological lesion. The unexposed control group had normal liver histoarchitecture while the exposed groups that were administered with 250mg/kg/BW of methanolic leaf extract of *Rauwolfia vomitoria* and *Aframomum melegueta* as well as the group co-administered with 250mg/kg/BW of both plants showed normal histoarchitecture of liver cells respectively with the sinusoids appearing normal, non-infiltrated or congested. The mRNA expression studies indicated an upregulation of IL-10 and NRF-2 in the studied organs. Generally, IL-10 expression was upregulated across all the treatment groups when compared to the control in rats treated with methanolic extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* at a standard dosage of 250mg/kg/BW with the most significant upregulation of IL-10 seen in the co-administrative treatment with both plants. Additionally, comparative analysis of the expression of IL-10 after administration of individual extracts was not significant. The significant elevation in IL-10 observed in rats co-administered with the plant extracts suggests a synergistic effect of both plants to induce IL-10 production further buttressing the traditional use of these plants in the local management of various ailments by traditional healers. The presence of alkaloids, particularly reserpine is suggested to be the potent component conferring the anti-inflammatory effect observed by the upregulation of IL-10 in the treated rats (Iyer and Cheng, 2012; Youmbie *et al.,* 2015). Reports on the RT-PCR analysis of Nrf- 2 revealed the same pattern observed in IL-10 expression in the co-administrative group as the most significant expression of Nrf-2 was evident in the co-administrative treatment group. However, unlike, the IL-10 expression where the single administration of the extracts does not significantly affect the expression of IL-10, we observed a significantly elevated expression of Nrf-2 in rats administered with methanolic extract of *Aframomum melegueta at 250mg/kg/bw when compared with rats administered with Rauwolfia vomitoria* at the same dosage. Studies by Okolie *et al.* (2011) documented that *Rauwolfia vomitoria stimulates the activity of* Nrf-2 which aligns with the findings in this study. Also, the presence of high flavonoids could have accounted for the observations in this study (Morakinyo *et al.,* 2019). The upregulation of Nrf-2 observed in rats coadministered with the plant extracts can be attributed to the combined activity of the phytochemical constituent of both plants which brings about a synergetic effect of the plants as NFR-2 activators as earlier reported by Morakinyo *et al*. (2020) documented a synergetic effect when *Aframomum melegueta* and *Piper guineense* was combined, with *Aframomum melegueta* having more effect due to its phytochemical constituent.

Conclusion

The findings in this study have reinforced the evidence from previous research that there is an immense range of therapeutic effects associated with *Rauwolfia vomitoria* and *Aframomum melegueta*, particularly anti-inflammatory and antioxidative properties that could be harnessed for their therapeutic purposes in drug development. In this study, there were no alterations in the histoarchitecture of the studied organs and administration at standard doses of 250mg/kg/bw for 28 days led to the upregulation of IL-10 and Nrf-2 in the treated rats. We therefore conclude that the administration of leaf extract of *R. vomitoria* and *A. melegueta* at the studied doses does not have a deleterious impact on the liver and kidneys of treated rats further suggesting their safety and justifying their usage in traditional medical practice.

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Author's contribution

TDA and MA were involved in the conception and planning of the research. MMA sourced for the plants and their herbarium numbers. FOA drafted the manuscript and designed the figures and tables. TDA and MA aided in interpreting the results. All authors took part in giving critical revision of the manuscript and have read and approved the final manuscript.

References

Adeniyi, T. D., Moronkeji. A. and Ekundina, V. O. (2023). Histological evaluation of the liver, kidney, and tests of adult male Wistar rats exposed to heavy metals-contaminated waterways. *Medical Laboratory Journal;* **17(5)**: 4-8.

Alo, M. N., Anyim, C., Igwe, J. C., Elom, M., and Uchenna, D. S. (2012). Antibacterial activity of water, ethanol, and methanol extracts of Ocimum gratissimum, Vernonia amygdalina and Aframomum melegueta. *Journal of Applied Sciences;* ***3*(2)**: 844–848.

Amole, O. O., Onabanjo, A. O. (1999). The effect and uses of *Rauwolfia vomitoria. Journal of Chemotherapy;* **3**:45-47.

Asoro, I.I., Ebuehi, O.A. and Igwo-Ezikpe, M.N. (2018). Comparative Toxicity Studies of *Rauwolfia vomitoria* Leaf and Root Extracts in Wistar Rats. *International Journal of Biochemistry Research & Review;* **22(2)**: 1-10

Bai, Y., Wang, X., Zhao, S., Ma, C., Cui, J., and Zheng, Y. (2015). Sulforaphane Protects against Cardiovascular Disease via Nrf2 Activation. *Oxidative Medicine and Cellular Longevity;* 2015: 407580.

Bare, Y. (2018). Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM). *Acta Informatica Medica;* ***26*(2)**: 87–92. https://doi.org/10.5455/aim.2018.26.87-92

Chiejina, N. V. and Ukeh J. A. (2012). Antimicrobial properties and phytochemical analysis of methanolic extracts of Aframomum melegueta and Zingiber officinale on fungal diseases of tomato fruit. *Journal of Natural Sciences Research;* ***2*(6)**: 10–16.

Coopoosamy, R., Singh, K., Naidoo, K. & Nadasan, D.S. (2023). 'The role of phytomedicine: Bridging the gap between the past, present, and future'. *Journal of Medicinal Plants for Economic Development;* **7(1)**: a197.

Ekong, M. B., Ekpene, U. U., Thompson, F. E., Peter, I., Udoh, N. B., & Ekandem, G. J. (2015). Effects of co-treatment of *Rauwolfia vomitoria* and Gongronema latifolium on neurobehaviour and the neurohistology of the cerebral cortex in mice. *Internet Journal of Medical Update;* ***10*(1)**: 3–10.

Eluwa, M. A., Udoaffah, M. T., Vulley, M. B. G., Ekanem, T. B., Akpantah, A. O., Asuquo, O. A., & Ekong, M. B. (2010). Comparative study of teratogenic potentials of crude ethanolic root bark and leaf extract of *Rauwolfia vomitoria* (apocynaceae) on the fetal heart. *North American Journal of Medical Sciences;* ***2*(12)**: 1–5.

Eteng, M. U., Ibekwe, H. A., Abolaji, A. O., Okoi, A. I., & Osuchukwu, N. C. (2009). Effect of *Rauwolfia vomitoria* Afzel (Apocynaceae) extract on serum amino transferase and alkaline phosphatase activities and selected indices of liver and kidney functions. *African Journal of Biotechnology;* ***8*(18)**: 4604–4607.

Ibrahim, R. B., and Onanuga, I. O. (2014). Sub-acute Toxicity of *Rauwolfia vomitoria* on the Kidneys and Liver of Wistar Rats: A Histological Study. *SMU Medical Journal;* ***2*(1)**: 166–177.

Imaga, N. O. A. (2010). The use of phytomedicines as effective therapeutic agents in sickle cell anemia. *Scientific Research and Essays;* ***5*(24)**: 3803–3807.

Iroghama, I., Osaretin, A. T., & Miriam, N. (2018). Comparative Toxicity Studies of *Rauwolfia vomitoria* Leaf and Root Extracts in Wistar Rats. *International Journal of Biochemistry Research & Review;* ***22*(2)**: 1–10.

Iyer, S. S., and Cheng, G. (2012). Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Critical Reviews in Immunology;* ***32*(1)**: 23–63.

Karnam, S., Girish, H. C., Murgod, S., Nayak, V. N., Varsha, V. K., & Yanduri, S. (2018). Rapid tissue processing technique: Anovel method using methyl salicylate. *Journal of Oral and Maxillofacial Pathology;* ***22*(3)**: 1–6.

Mensah, M.L., Komlaga, G., Forkuo, A.D., Firempong, C., Anning, A.K. and Dickson, R.A. (2019). Toxicity and safety implications of herbal medicines used in Africa. *Herbal Medicine;* ***63*(5)**:1992-0849.

Morakinyo A.E., Fasakin W.O., Fajobi A.O., Akinpelu B.A., Oyedapo O.O. (2020). Effects of Aframomum melegueta leaf extract on the antioxidant status of obese rats. *African Journal of Biology and Medical Research*; **3(3)**: 21–31.

Morakinyo, A.E., Oyedepo, T.A., Babarinde, O., Adelowo, J.M., Adepoju, A.E. and Oyedapo, O.O. (2019). Anti-hyperglycemic and anti-hyperlipidemic potentials of methanol leaf extracts of Aframomum melegueta and Piper guineense. European *Journal of Medicinal*

Plants; **27(1)**: 1–8.

Mothibe, M.E. & Sibanda, M. (2019). African traditional medicine: South African perspective, in C. Mordeniz (ed.), Traditional and Complementary Medicine, IntechOpen: 1 – 27.

National Academy Sciences. (2011). Guide for the Care and Use of Laboratory Animals. Institute for Laboratory Animal Research. 8th ed. Washington, DC: National Academies Press: 60.

Obike, H. I., Ezejindu, D. N., Akingboye, A. J., & Ezejindu, C. N. (2014). The Effects of Aframomum melegueta Aqueous Extract on the Liver of Adult Wistar Rats. *Internal Journal of Advances in Pharmacy, Biology and Chemistry; **3*(2)**: 329–333.

O'Garra, A., Barrat, F. J., Castro, A. G., Vicari., A., and Hawrylowicz, C. (2008). Strategies for use of IL-10 or its antagonists in human disease. *Immunology Review;* **223**:114–131.

Okolie, N. P., Israel, E. E. J., and Falodun, A. (2011). In-vitro evaluation of antioxidant potential of *Rauwolfia vomitoria* root extract and its inhibitory effect on lipid peroxidation as indication of aphrodisiac properties. Pharmaceutical Chemistry Journal*;* **45(8)**: 476–480.

Osuntokun, O. T. (2020a). Aframomum Melegueta (Grains of Paradise). *Annals of Microbiology and Infectious Diseases; **3*(1)**: 1–6.

Osuntokun, O. T. (2020b). Aframomum Melegueta (Grains of Paradise). *Annals of Microbiology and Infectious Diseases; **3*(1)**: 1–6.

Oyewole, O., and Massaquoi, H. (2019). Oral administration of *Rauwolfia vomitoria* extract has no untoward effect on kidney and liver functions in rats. *International Journal of Histology and Cytology; **6*(6)**: 1–4.

Peter, A. I., & Ekpene, U. (2021). Co-administration of *Rauwolfia vomitoria* with Gongronema latifolium or Vernonia amygdalina on spatial learning, memory, and some biomolecules. *Asian Journal of Medical Sciences; **7*(1)**: 82–87. https://doi.org/10.3126/ajms.v7i1.11156

Rashid, S., Majeed, L.R., Nisar, B., Nisar, H., Bhat, A.A. & Ganai, B.A., 2021, 'Phytomedicines: Diversity, extraction, and conservation strategies', in R.A. Bhat, K.R. Hakeem & M.A. Dervash (eds.), Phytomedicine: A treasure of pharmacologically active products from plants. Academic Press, Cambridge, MA: 1–33.

Sahin, K., Tuzcu, M., Orhan, C. Gencoglu, H., Ulas, M., Atalay, M., Sahin, N., Hayirli, A., and Komorowski, J. R. (2012). The Effects of Chromium Picolinate and Chromium Histidinate Administration on NF-κB and Nrf2/HO-1 Pathway in the Brain of Diabetic Rats. *Biological Trace Element Research;* **150:** 291–296.

Shing, C., Woo, J., See, J., & Lau, H. A. N. (2019). Herbal Medicine: Toxicity and Recent Trends in Assessing Their Potential Toxic Effects. *School of Biological Sciences, Faculty of Science, The University of Hong Kong, Hong Kong SAR, China.* 1st ed., Vol. 62.

Steen, E.H., Wang, X., Balaji, S., Butte, M.J., Bollyky, P.L. and Keswani, S.G. (2020). The role of the anti-inflammatory cytokine interleukin-10 in tissue fibrosis. *Advances in Wound Care; **9*(4)**:184-198.

Umukoro, S., and Aladeokin, A. C. (2011). Therapeutic Effects of Grains of Paradise (Aframomum melegueta) Seeds. *In Nuts and Seeds in Health and Disease Prevention.* Academic Press: 535– 543.

Youmbie, D. D. B., Dzeufiet, D. P. D., Nkwengo, E., Mezui, C., and Dimo, T. (2015). Antiinflammatory and Anti-nocieptive effects of the stem bark aqueous extract of *Rauwolfia vomitoria. World Journal of Pharmacy and Pharmaceutical Sciences; **4*(7)**: 90–112.

Yunes, R. A., Niero, R., & Filho, V. C. (2012). Medicinal Plants and Phytomedicines. *Journal of Phytochemistry and Pharmacognosy; **88**:* 302–202.

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https://dx.doi.org/10.4314/sokjmls.v9i2.24