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An Efficacy Study On The Effect Of Agbo Iba Herbal Decoction And Acetaminophen Administrations On Pro-Inflammatory And Nociception In Male Wistar Rats After Freund's Incomplete Immune Activation (FIA)

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Abstract **Original Research Article**

Acetaminophen (paracetamol) is a widely used analgesic and antipyretic, but its narrow therapeutic index raises concerns particularly when co-administered with herbal remedies like Agbo Iba in inflammatory states. This study investigated the effects of Agbo Iba (500 mg/kg), acetaminophen (300 mg/kg), and their combination on nociception, pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) parameters in male Wistar rats under Freund's Incomplete Adjuvant (FIA)-induced immune activation.Phytochemical analysis revealed Agbo Iba is rich in alkaloids (39.54 mg/100 g), phenolic compounds (23.30 mg/100 g), and flavonoids (20.83 mg/100 g). The LD50 of Agbo Iba exceeded 5000 mg/kg, indicating relative safety. The combination of Agbo Iba and acetaminophen produced the strongest analgesic effect (78–80% inhibition) the acetic acid-induced writhing test, outperforming individual treatments. FIA elevated pro-inflammatory cytokines; combined therapy significantly attenuated TNF-α, IL-1β, and IL-.Sequential co-administration of Agbo Iba and acetaminophen offers enhanced anti-nociceptive and anti-inflammatory benefits under immune activation but warrants caution regarding potential liver interactions. These findings support further standardization and clinical studies on herb-drug interactions in traditional medicine practices.

Keywords: Agbo Iba, acetaminophen, Freund's Incomplete Adjuvant, inflammation, nociception, Wistar rats.

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INTRODUCTION

Acetaminophen (paracetamol) is one of the most commonly used antipyretic and analgesic agents worldwide, but its therapeutic index is narrow, and its metabolism is highly sensitive to hepatic oxidative stress and inflammatory states (Ohashi *et al* ., 2020). The risk of hepatotoxicity increases when acetaminophen is administered alongside substances that modulate cytochrome P450 enzymes, glutathione levels, or pro-inflammatory cytokines. Traditional polyherbal preparations such as Agbo Iba, widely used in Nigeria for fever, malaria, and inflammatory conditions, contain multiple phytochemicals capable of altering antioxidant pathways, hepatic enzymes, and immune responses (Nwabuisi *et al* ., 2002; Erhunse *et al*., 2023).

However, the combined effect of Agbo Iba and acetaminophen remains poorly characterized, especially under conditions of immune activation.

Despite the widespread use of Agbo Iba in febrile Freund's Incomplete Adjuvant (FIA) is known to induce localized inflammatory responses and mild systemic immune stimulation, including upregulation of cytokines and enhancement of antigen processing (Billiau & Matthys, 2001). Immune activation is a critical modifier of drug metabolism because inflammatory cytokines can suppress hepatic enzyme activity and alter pharmacokinetics, potentially intensifying drug-induced liver injury. The interaction between herbal mixtures and acetaminophen may therefore be significantly different in the presence of immune stimulation from FIA.

and inflammatory states—conditions where acetaminophen is also commonly used—there is a lack of experimental studies evaluating the co-administration of these agents under an activated immune environment. This gap limits the ability The combined of Agbo–paracetamol sequential-administration and usage is a common practice in Nigeria notably at West-North region of Nigeria. Some of these practices have even been seen in the South-East Region of Nigeria, largely because of the belief that such combination relieves one of malaria, fever and the symptoms that come with it. Paracetamol is the most common used Acetaminophen, which is why it will be used for this study. It presents its own challenges, most notably on the liver, though there is evidence of toxicity on other tissue. A limited number of researches have been carried out on Agbo Iba especially in a model involving a challenging health situation mimic like in Freund's Incomplete Adjuvant Stimulation to better represent a study on systemic inflammatory activation in different systems like those of the reproductive functions (notably since there are global concerns on healthy population growth index) .This study not only presents that situation but creates an investigative platform for its interaction with Acetaminophen. The result from this study will further elucidate how paracetamol actually works alone and interacts with the herbal decoction in an inflammatory situation. This work will be useful to researchers, orthodox and modern medicine in planning dosages, usages, purification, extraction and therapies of Agbo Iba with or without Acetaminophen.

MATERIALS AND METHOD

A Total of fifty-four (54) Male Wistar rats, housed under standard conditions, were used for the main study. They were 8–10 weeks old with weight range of 200–250 g. They were obtained from the laboratory animal production unit of the Department Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike. They were used for the different segments of the study. Additional Twenty (20) rats will be used for sub toxicity study of Agbor. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of 25±2oC and were fed with rat chow (Grand Cereals Ltd, Abia State). They had access to clean drinking water for seven days of acclimatization into the initiation of experiment. All experiments were carried out in accordance with International Guideline for Biomedical Research and as approved by the Department of Physiology and University Ethical Committee in Abia State University, Uturu.

Inclusion and Exclusion Criteria

The animals were examined for the following criteria to determine involvement in the study:

1. Sex and species: Only male Wistar rats (*Rattus norvegicus*) were used for this study
2. Weight: Rats weighing between 200-250 grams at the start of the experiment were used for this study
3. Drug Exposure: Only rats with no prior drug exposure were included in the study to prevent interference with experimental outcomes.
4. Acclimatization: only rats that have undergone an acclimatization period of at least 7 days under standard laboratory conditions before treatment began, were used for this study.

5. Viability: Rats with physical inflamed damage prior or after acclimatization were not used for this study. This is to reduce error in result as the work involves induction of mild systemic inflammation Experimental induction Design

Induction of Incomplete Freund's Adjuvant (FIA) was in accordance with previously published method (Kolstad, *et al*, 2012). Incomplete Freund's Adjuvant (FIA) was administered via **subcutaneous injection**. Each rat will receive **0.1 mL of FIA per injection site**, with injections delivered at the **base of the tail** to produce localized mild inflammation while minimizing systemic toxicity. Acetaminophen and *Agbo Iba* was administered orally in their respective doses a day following FIA injection and daily thereafter for the study duration. This approach is designed to produce a measurable pain-like response without inducing severe arthritis, thereby enabling the evaluation of the analgesic and protective effects of the test agents. The induction of FIA was used to mimic the chronic inflammation involving pyrogenic (fever-causing) cytokines such as TNF-α and IL-6 (Kolstad, *et al*, 2012, Billiau and Matthys, 2001)

The Agbo Iba Preparation

Agbo Iba is a standard decoction of neem leave or dogonyaro (*Azadiracta Indica*), mango bark (*mangifera indica*), guava (*Psidium guajava*) leaves, lemon grass (*Cymbopogon citratus*) and bitter leaf (*vernonia amygdalina)*. The preparation doesn't have a standardize herbs, dosage or concentration, even though some herbs are common in the preparations. In addition, the selected plant was in accordance with the work of Iyama and Idu (2017). This w was selected because the plant component could be found almost anywhere within the region.

The polyherbal decoction was prepared based on recommendations including that of World Health Organization for Herbal Preparations (Monaga, *et al* ., 2022; WHO, 2018). The plants parts were freshly collected and dried under the sun (to avoid denature that may come from dryer or oven heat). They were mixed in 1:1:1:1:1 mass ratio and grinded to powder. In the powder form, they were soaked in water (ethanol is a better solvent for extraction but it was not used so as to avoid false results due to ethanol toxicity). The mixture was boiled for 30 minutes. The extract was concentrated and dried to obtain a measurable yield for administration. This was adopted to better utilize the active ingredients (as ethanol was not used).

Determination of Sub-acute toxicity (LD50) effect of the extracts

The acute toxicity of the each extract was determined in rats using the method of Lorke (1983) as reported by Orieke et al (2019). The tests involve two phases. In the first phase, 9 rats assigned to 3 groups were given 10, 100 and 1000 mg/kg body weight oral doses of the extract. The death or survival records in first phase was determined the doses used for the second phase. In the second phase, another set of 9 rats also assigned to 3 groups of 3 rats each were treated with 1600, 2900 and 5000 mg/kg body weight of the extract respectively if death doesn't occur in the first phase. The treated animals were observed for 24hours and a further 7 days for signs of toxicity and lethality.

The lethal dose of the herbal extract was found to be greater than 5000mg/kg. 500mg/kg of the herbal extract was selected for comparison of efficacy and not doses. The dose of Agbor represents only one-tenth of the 5000mg/kg for which its lethal dose is above. In addition, doses within this range are commonly employed in experimental studies involving crude plant extracts.

Acetaminophen and Vitamin C Administration

Acetaminophen and white Vitamin C tablet will be bought from a pharmacy in Umuahia, Abia State. They will be concentrated as 10mg/ mil of water. A dose of 300mg/kg of Acetaminophen and 100mg/kg of vitamin C will be for the groups and positive group respectively. Administration of Agbo Iba, Acetaminophen and Vitamin C will be for 14 days. These dose were used to reflect a dose for 14 days study of notable biochemical effect with no record of mortality (Yakubu, *et al*., 2013, Akinmoladun, *et al*., 2017, Ezeja, *et al.*, 2015)

Administration Sequence and Animal use

To mimic human administrative pattern, and to maximize interaction, Agbo Iba were given first. 30 minutes later, Acetaminophen given after which Vitamin C (100 mg/kg) administered.

Table 1a: Groups of the Experimental animals with treatments of each groups

Group	Group Name	Treatment/Drug Administered
Group 1	Vehicle control	Normal Saline
Group 2	Negative control	Freund's Incomplete Adjuvant (FIA)
Group 3	Positive Control	FIA + Vitamin C (100mg/kg/ day)
Group 4	Acetaminophen group	Acetaminophen (300mg/kg/day)
Group 5	Agbo Iba group	Agbo Iba
Group 6	Mixed Acetaminophen group	Acetaminophen (300mg/kg/day) + Agbo Iba
Group 7	Adjuvant Agbo group	FIA + Agbo Iba
Group 8	Mixed Adjuvant Acetaminophen group	FIA + Acetaminophen (300mg/kg/day)
Group 9	Mixed Adjuvant Treament group	FIA + Acetaminophen (300mg/kg/day) + Agbo Iba

n= 6

At the end of treatment, animals were assessed for nociception using acetic induced writhing test after which they were sacrificed and blood samples collected into EDTA and plain bottles for biochemical analysis. For the later (blood contained in plain bottles), the blood was allowed to clot and retract within two hours and then centrifuged at 3000 rpm for 10 minutes to obtain clear sera samples which were used for the various biochemical analyses of biomarkers of inflammation and other functions.

Ethical Clearance

Ethical clearance was sought and obtained from the University Ethical Committee, Directorate of Research and Publications, Abia State University, Uturu.

Statistical Analysis

Statistical analysis was performed with IBM SPSS version 23. The results were determined using One Way Analysis of Variance (ANOVA), followed by student Newman- Keul's Post-hoc test, complemented by Duncan Mulitple Range Test. Data was presented in tables, and as mean ± standard error of mean (SEM) where applicable. Mean of Values at $P < 0.05$ was considered statistically significant.

RESULTS OF THE STUDY.

The result of the physiological and biochemical parameters were analyzed and comparisons were reported on the significant level of $P<0.05$ as significant and $P>0.05$ not significant.

Table 1a: Qualitative and Quantitative phytochemical contents of Agbo-Iba Herbal decoction

Phytochemicals	Inference	Quantitative availability (mg/100 g)
Saponins	++	16.17±0.19
Tannins	++	6.86±0.56
Phenolic compounds	+++	23.30±0.93
Flavonoids	+++	20.83±0.75
Steroids	+	3.80±0.16
Terpenoids	+	4.04±0.10
Glycosides	+	3.07±0.09
Alkaloids	+++	39.54±0.89

Values are as mean ± standard deviation (n = 6). Keys: + = Present, ++ = more present, +++ = most present.

The result of the phytochemical analysis in this study showed that the herbal preparation, Agbo Iba is rich in Alkaloids, Phenolic compound and flavonoids in decreasing order of content. It is mildly rich in saponins. Alkaloids have shown to possess analgesic, anti-inflammatory and anti-malaria effects especially quinines (Radulović, *et al* ., 2013; Sireesha, *et al* ., 2019; Barbosa-Filho, *et al* ., 2006,).

Phenolic Compounds are well known for their antioxidant and anti-inflammatory effects with significant immunomodulatory properties (Zhang, *et al* ., 2022; Yahfoufi, *et al*., 2018) Favonoids just like phenolic compounds exhibit antioxidant, anti-inflammatory and immunomodulatory effects especially on inflammatory cytokines such as Tumor necrosis Factor (TNF-α), Interleukin -1βeta (IL-1β) and Interleukin-6 (IL-6)

Table 1b: Quantitative phytochemical content of Agbo-Iba formular

Phytochemicals	Quantitative availability (mg/100 g)
Saponins	16.17±0.19
Tannins	6.86±0.56
Phenolic	23.30±0.93
Flavonoids	20.83±0.75
Steroids	3.80±0.16
Terpenoids	4.04±0.10
Glycosides	3.07±0.09
Alkaloids	39.54±0.89

Values are as mean ± standard deviation (n = 6)

Table 2 showing Results of lethal dose evaluation (LD_{50}) of Agbo Iba herbal Decoction Phase 1

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

Table 2b showing Results of lethal dose evaluation (LD_{50}) of Agbo Iba herbal Decoction Phase 11

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5000	0/3	Animals were calm for about one hour, but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

LD_{50}> 5000 mg/kg body weight

The result of the lethal dose test showed that the herbal decoction of Aqueous Agbo Iba is safe for consumption up to 5000mg/kg of body weight. This shows that it is relatively safe for consumption.

Table 3 showing Results on the Effect of Agbo Iba herbal Decoction and Acetaminophen administrations on Acetic acid-induced writings of Male Wistar rats after Freund's incomplete Immune activation (FIA).

Treatment groups	Number of writhes in 30 minutes	Percentage inhibition of pain
Normal Control	67.67 ± 3.79^{d}	0.00 ± 0.00^{c}
Freund's Incomplete Adjuvant (FIA) only	84.33 ± 5.03^{e}	-25.87 ± 7.51^{a}
FIA + Vitamin C (100 mg/kg bw)	74.00 ± 3.61^{d}	-10.44 ± 5.38^{b}
Acetaminophen (300 mg/kg bw) only	23.00 ± 3.00^{b}	65.67 ± 4.48^{e}
Agbo Iba (500 mg/kg bw) only	$19.00\pm3.00^{a,b}$	$71.64\pm4.48^{e,f}$
Acetaminophen (300 mg/kg) + Agbo Iba (500 mg/kg)	13.67 ± 2.31^{a}	79.60 ± 3.45^{f}
FIA + Agbo Iba (500 mg/kg bw)	34.67 ± 5.51^{c}	48.26 ± 8.22^{d}
FIA + Acetaminophen (300 mg/kg bw)	29.67 ± 4.04^{c}	55.72 ± 6.03^{d}
FIA + Acetaminophen (300 mg/kg bw) + Agbo Iba (500 mg/kg bw)	14.67 ± 1.16^{a}	78.11 ± 1.72^{f}

Values are presented as mean ± standard deviation (n = 6); and values with different letter superscripts are significantly ($P < 0.05$) different within the column.

The acetic acid-induced writhing test is a highly sensitive experimental model of visceral nociception in which abdominal constrictions (writhes) are quantified as an index of pain perception. Increased writhing frequency indicates enhanced nociception or hyperalgesia, while reductions in writhing are indicative of analgesic activity. Despite its high sensitivity, the test is considered relatively non-specific because the response involves multiple inflammatory mediators, including prostaglandins, cytokines, mast cells, and macrophages (Koster *et al* ., 1959; Ribeiro *et al* ., 2000; Dzoyem *et al* ., 2017).

Freund's incomplete Immune Activation contains mineral oil which is reported to strongly activate leucocytes, dendritic cells, TLR2/TLR4 pathways and NF-κB signaling (Kolstad, *et al* ., 2012; Zamora, *et al* ., 2002, Kohashi, *et al* ., 2001). These result to systemic inflammatory responses such as hyperalgesia and oxidative stress with notable nociceptive behavior (Noh, *et al* ., 2021)

The result of nociception showed that the sequential therapy of acetaminophen and Agbo iba after FIA induction, showed the lowest writhes when compared to all the groups at ($P<0.05$). Though there was no significant difference in writhes between single administrations of Agbo Iba and Acetaminophen, they both significantly decreased the number of writhes even compared to the normal control. The analgesic effect of acetaminophen is well established. However, the analgesic effect of Agbo Iba, which is almost same with acetaminophen may be due to its high content of Alkaloids which exhibit strong analgesic effects by acting directly on pain pathways. Their action result to opioid receptors blockage, sodium channel blockade (nerve conduction inhibition), central nervous system depression/modulation and NMDA receptor modulation (Aly, *et al* ., 2025; Ferraz, *et al* ., 2020; Radulovic, *et al* ., 2013)

Except for the Freund activation group, Vitamin C showed the highest writhes; an indication that it may have low analgesic activity against FIA, probably

because its anti-nociceptive effect is considered secondary to its anti-inflammatory effect when compared to acetaminophen (Lu, *et al* ., 2011; Mallet, *et al* ., 2010)

Table 4 showing Results on the Effect of Agbo Iba herbal Decoction and Acetaminophen administrations on Pro-inflammatory cytokines of Male Wistar rats after Freund's incomplete Immune activation (FIA).

Treatment groups	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
Normal Control	19.96±2.45^{b}	0.85±0.08^{a,b}	4.30±0.23^{b}
Freund's Incomplete Adjuvant (FIA) only	51.43±3.35^{f}	1.82±0.24^{d}	9.46±1.61^{d}
FIA + Vitamin C (100 mg/kg bw)	37.00±3.14^{e}	1.30±0.05^{c}	5.57±0.23^{c}
Acetaminophen (300 mg/kg bw) only	15.24±0.76^{a,b}	0.65±0.11^{a}	3.07±0.37^{a}
Agbo Iba (500 mg/kg bw) only	15.78±3.83^{a,b}	0.65±0.12^{a}	3.16±0.12^{a}
Acetaminophen (300 mg/kg) + Agbo Iba (500 mg/kg)	14.60±2.69^{a}	0.69±0.13^{a}	2.17±0.22^{a}
FIA + Agbo Iba (500 mg/kg bw)	30.97±1.94^{d}	1.05±0.09^{b}	5.80±0.31^{c}
FIA + Acetaminophen (300 mg/kg bw)	25.04±2.47^{c}	1.08±0.15^{b}	5.65±0.86^{c}
FIA + Acetaminophen (300 mg/kg bw) + Agbo Iba (500 mg/kg bw)	17.23±2.86^{a,b}	0.87±0.07^{a,b}	5.09±0.23^{c}

Values are presented as mean ± standard deviation (n = 6); and values with different letter superscripts are significantly (P < 0.05) different within the column. TNF-α – Tumor necrosis Factor, IL-1β- Interleukin -1βeta

The result showed that the Freund's Incomplete Adjuvant activation (FIA) caused the highest significant increases in pro-inflammatory cytokines as these increases point to tissue injury, immune activation, inflammation, fever and pain sensitization (Kolstad, *et al* ., 2012; Noh, *et al* ., 2021). Though single administrations of acetaminophen and the herbal decoction (both without FIA), significantly decreased the levels of the pro-inflammatory cytokines, there was no significant difference between them. After FIA induction, Acetaminophen significantly reduced the levels of TNF-α when herbal decoction in

The combined sequential therapy of Acetaminophen and Agbo Iba , after FIA induction produced similar significance (P<0.05) of reduced TNF-α levels when compared to individual groups treated with Acetaminophen and Agbo Iba.

The sequential therapy of acetaminophen and Agbo Iba, without FIA induction significantly reduced the serum levels of IL-6 as compared to those

However, levels of IL-6 were significant reduced, especially in the group co-treated with acetaminophen and Agbo Iba when compared to all other groups indicating that Combined administration of Acetaminophen and Agbo Iba may exhibit stronger anti-inflammatory and immune-suppressing effect than individual single administrations Several works as earlier stated, have shown how FIA can be used to induce chronic local inflammation of pyrogenic (fever-causing) Pyrogens such as TNF-α and IL-6 (Kolstad, *et al* , 2012, Billiau and Matthys, 2001). The result of this study showed Acetaminophen and Agbo Iba should exert more

anti-inflammatory effects than when only one in cases of fever

CONCLUSION

This study demonstrates that sequential administration of Agbo Iba and acetaminophen provides enhanced antinociceptive and anti-inflammatory benefits in FIA-induced inflammation compared to individual agents, stressing the importance of combined therapy in medical sciences. These findings have practical implications for traditional medicine practitioners and clinicians in regions where Agbo Iba and paracetamol are commonly used. Future studies should include histopathological examination, dose optimization, full herbal standardization (e.g., via HPLC), larger sample sizes, and long-term safety assessments. This work contributes to the evidence base for safe integration of herbal remedies with conventional analgesics.

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