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Modulatory Activity of *Aju Mbaise* Polyherbal Formulation on Serum Proinflammatory Cytokines and Prostaglandin Levels in Nulliparous and Parturient Female Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aju Mbaise (*Ajumbise*), which traces its origin to the people of Mbaise in the Owerri Senatorial District of Imo State in Nigeria, is a composite mixture of approximately eight herbal plants in the forms of herbs, barks, and roots, used for the management of delivery pains, postpartum associated disorders and facilitate recoil of the uterus after delivery. In this study, the modulatory

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effects of different solvent extracts of the polyherbal on inflammatory markers were investigated in parturient and non-pregnant rats. The rats which were assigned to ten groups of 5 rats each (with controls) were treated such that each group was administered a specific solvent extract (500 mg/kg body weight) while the control groups received normal saline. Treatment lasted 28 days before animals were sacrificed for sample collection and analyses of pro-inflammatory markers. Results of the lethal dose (LD_{50}) assessment of the extracts revealed values >5000 mg/kg body weight for all four extracts. The serum concentrations of interleukin-1b, interleukin-6 and prostaglandin E2 were significantly reduced in the treated nulliparous and parturient rats for all extracts when compared with their respective control values ($p<0.05$), but that of prostaglandin E1 increased across the test groups when compared with their controls ($p<0.05$). Therefore, ethanol, chloroform, diethyl ether and aqueous extracts of *Ajumbise* polyherbal formulation may be safe modulatory agents for the management of pain and inflammatory disorders usually associated with elevated inflammatory cytokines levels and may be used as such, especially in females.

Keywords: Ajumbise polyherbal formulation; interleukin-1b; interleukin-6; nulliparous; parturient; prostaglandin E2.

1. INTRODUCTION

Herbal, alternative or complementary medicine is currently gaining general acceptance as a good strategy for the management of diseases [1]. The World Health Organization (WHO) had reported that 60% of the world's population relies on herbal medicine and about 80% of the population in developing countries depends almost totally on it for their primary healthcare needs (Khan et al. 2019). The rising demand for herbal medicaments may be due to their perceived safety profile, cost-effectiveness, strength, effectiveness, and eco-friendly therapeutics potential [1]. Polyherbal formulations have indeed inspired a paradigm shift in the use and applications of herbal medicines (Chauhan et al., 2015; Feinberg et al., 2019). *Ajumbise* is a Nigerian polyherbal formulation which originates from the Mbaise people of Imo state and is consumed by parturients for the purpose of treating delivery-related pains, and postpartum-associated disorders and facilitating recoil of the uterus after delivery. Non-pregnant females consume Ajumbise to achieve weight loss and to treat fibroid and other obstetric conditions.

There is also a claim that Ajumbise induces abortion in pregnancy, hence pregnant women are cautioned against the use of the agent (Westfall, 2001; Foster et al., 2006, Holst et al., 2008; Adams et al., 2009, Hall et al., 2012; Palivalappila et al., 2014; Palivalappila et al., 2015), even though findings made by Ijioma et al. (2020) strongly disagree with the claim. The plant composition of *Ajumbise* polyherbal formulation appears to be a subject with different opinions. For example, Ijioma et al. [2], identified six plants including *Euphorbia convolvuloids*, *Uvaria chamae*, *Spondias mombine*, *Ceiba petandra*, *Barteria fistulosa* and *Napaloena vogelli* as the herbal components of *Aju Mbaise*, where as Iwueke and Chukwu, (2020) in their own study identified and named nine (9) plants namely *Cnestis ferruginea*, *Xylopia aethiopica*, *Uvaria chamae*, *Palisota hirsute*, *Scteria Spp*, *Napoleonea imperialis*, *Dialium guineense*, *Combretum racemosum* and *Heterotis rotundifolia* as its component plants. A closer look reveals that the commonality in constituents shared by the authors is the plants *Uvaria chamae* and *Napoleonea Spp*. These differences further affirm the fact that plant identification inconsistencies remain a significant source of concern in ethnomedicinal research (Tunde, 2019).

Some pharmacological activities have been linked to individual plants in *Ajumbise* polyherbal. Abortifacient activities have been attributed to *Spondias mombin* following studies carried out in laboratory albino rats (Akah, 1994; Nworu et al., 2007). The aqueous extract of *Euphorbia convolvuloids* reportedly exhibited significant anxiolytic, analgesic, antipyretic, and anti-inflammatory activities in rats [3]. The bacteriostatic and bactericidal activities of *Uvaria chamae* have also been reported [4]. Although the literature on the pharmacological potentials of *Ajumbise* polyherbal formulation remains very scanty, few works have been carried out on the herbal preparation and reported. The insignificant activity of the formulation on uterine contractility and contractile effects of its *Uvaria chamae* component has been reported [2]. Other reported activities are its anti-motility effect and usefulness in diarrhoea management (Ijioma et

al., 2019), Anti-red blood cell fragility and antioxidant effects [5] and its toxicity [6] and phytochemical compositions [6].

Pain complaints, in the immediate postpartum period, are suffered by most puerperal women (about 90%) globally regardless of the mode of delivery and parity. Cytokines are known inflammatory mediators. The cytokines, IL-1b and IL-6, are known proinflammatory markers. Studies have established the role of PGE2 in tissue regeneration. PGE1 is reported to stimulate IL-6 secretion. Perinatal pain management has remained a topical issue. Studies have established a connection between labour and postpartum pain with postpartum depression as a thematic issue in maternal morbidity. (Cheng et al., 2021; Mathur et al., 2021; Pereira et al., 2017; Watanabe-Tomita et al., 1997) *Aju Mbaise* polyherbal formulation is acclaimed to relieve pain and improve well-being postpartum [6]. The mechanism is not known. The current study aimed to evaluate the modulatory effects of *Ajumbise* polyherbal formulation on serum concentrations of pro-inflammatory cytokines in non-pregnant and parturient rats.

2. MATERIALS AND METHODS

2.1 Quantitative Phytochemical Determination of the Extracts

Qualitative and quantitative determination of phytochemical agents in the extract was carried out in accordance with the methods described by Harborne, (2005) while quantification of bioactive compounds by gas chromatography-mass spectrometry (GC-MS) was done following the method described by Igwe et al. (2014).

2.2 Acute Toxicity (LD_{50}) Evaluation of *Ajumbise polyherbal* Extracts

For the LD_{50} evaluation of each solvent extract of Ajumbise polyherbal, a modified Lorke's method used by Orieke et al., (2019) was adopted. The protocol was carried out in 3 stages and involved the use of 21 albino rats. In the first stage, 9 rats assigned to 3 groups (1, 2 and 3) were administered 10, 100 and 1000 mg/kg single oral doses of the extract respectively and were thereafter placed under observation within 24 hours for toxicity signs including death. The study proceeded into the second stage when zero per cent mortality was observed across the groups. In the second stage, another set of 9 rats also assigned to 3 groups of 3 rats each were administered 1600, 2900 and 5000 mg/kg of the extract respectively and were also observed as earlier mentioned. Following the observance of zero percent mortality across the groups, the highest dose (5000 mg/kg) was repeated on another set of 3 rats as a confirmatory test. This last set of rats was observed within 24 hours and a further 7 days stage.

The acute toxicity value of the extract was calculated using Lorke's formula below:

$LD_{50} = \sqrt{A\ x\ B}$

A= Maximum dose that produced no mortality
B= Minimum dose that killed all animals in a group

2.3 Experimental Animals and Design for the Evaluation of the Effect of the Extracts on Serum Cytokine Levels in Female Rats

A total of 50 (25 nulliparous and 25 parturient) inbred female Wistar rats weighing 140-180 g obtained from the laboratory animal production unit of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike were used for the study. The animals were housed in clean aluminum cages, fed *ad libitum* with Chikkun finisher's mash and clean water and acclimatized for two weeks before commencement of the experiment. Each set of rats was assigned to 5 groups of five rats each and treated as shown below:

Group A: 500mg/kg body weight of aqueous extract
Group B: 500mg/kg body weight of ethanol extract
Group C: 500mg/kg body weight of diethyl ether extract
Group D: 500mg/kg body weight of chloroform extract
Group E: 0.2 ml normal saline and served as control.

Treatments were oral and lasted 28 days before animals were sacrificed for sample collections. The body weights of the rats were measured at the beginning and end of the study using an electronic weighing balance (Model p300, SANFA scientific instruments, Shanghai, China).

2.4 Collection of Blood Samples and Determination of Inflammatory Cytokines Levels

Blood was collected from each animal by cardiac puncture into plane bottles following chloroform anaesthesia. The collected samples were later centrifuged at 3000 rpm for 10 minutes to obtain clear sera which were subjected to pro-inflammatory cytokine levels tests. The cytokines tested for were interleukin-1b, interleukin-6 and prostaglandin E2. Serum levels of interleukin-1β (IL-1β), interleukin 6 (IL-6) and PGE2 were determined using ELISA kits with catalog numbers CSB-E08055r, E04640r and CSB-E07967r respectively, produced by CUSABIO laboratories, China while Prostaglandin E1 (PGE1) concentration was determined in the collected sera using ELISA Kit (Catalog # E4716-100) produced by BioVision incorporated, USA. Standard procedures prescribed by producers of the ELISA kits for each test were carefully adhered to.

2.5 Statistical Analysis

Results were presented as mean values ± standard deviations (mean ± SD) using. The replicates in each treatment were subjected to a one-way analysis of variance (ANOVA) and the difference between the sample's mean was tested by Tukey post-hoc test using R-statistics software version 3.03. P-values ≤ 0.05 were considered as being statistically significant.

3. RESULTS

3.1 Results of Phytochemical Composition of *Ajumbise* polyherbal Extract

Among the phytochemical agents identified in the four extracts were tannins, flavonoids, saponins, steroids, terpenes, alkaloids, cardiac glycosides, and phenolic compounds. The quantities of these identified phytochemicals also differed relatively, as some were found in higher amounts while others were present in only trace amounts. Higher amounts of the abundant phytochemicals (Alkaloids, flavonoids, phenols and saponins) were present in ethanol and aqueous extracts when compared with amounts present in chloroform and diethyl ether extract. The values obtained for these phytochemicals in the four different solvent extracts of Ajumbise polyherbal extract are presented in Table 1.

3.2 Effects of *Ajumbise* Polyherbal Extract on Pro-inflammatory Cytokines (PGE1, PGE2, IL-6 and IL-1b) in Nulliparous Rats

On nulliparous female rats, treatment with chloroform, diethyl ether and aqueous extracts significantly lowered serum concentrations of prostaglandin E2 when compared with a control value ($p<0.05$), while the group treated with ethanol extract had serum PGE2 concentration which did not significantly differ from that of the control ($p>0.05$) even though serum PGE1 concentration in this group was significantly higher than that of control ($p<0.05$). Serum levels of PGE1 in groups treated with chloroform and diethyl ether were not significantly altered when compared with the control ($p>0.05$). The effect of the aqueous extract on serum levels of PGE1 in the nulliparous rats followed the same pattern as that of the ethanol extract. While serum levels of IL-1b were significantly lowered across all treatment groups, those of IL-6 were only significantly lowered in groups treated with ethanol, chloroform, and aqueous extracts ($p<0.05$). Serum IL-6 concentration was not significantly altered in the group treated with diethyl ethyl ether extract. Results showing the effects of the extracts on the levels of these pro-inflammatory markers are presented in Table 2.

3.3 Effects of *Ajumbise* Polyherbal Extract on Pro-inflammatory Cytokines (PGE1, PGE2, IL-6 and IL-1b) in Parturient Rats

In the parturient rats, treatment with all the extracts significantly lowered serum concentrations of PGE2 and increased those of PGE1 when compared with the control ($p<0.05$). The highest lowering effect on PGE2 was observed in the chloroform extract-treated group while the highest increase in PGE1 was observed in the aqueous extract-treated group. The serum level of IL-6 was only significantly lower than that of the control group treated with the ethanol extract ($p<0.05$). Results obtained also showed that serum IL-1b concentration was significantly reduced across all extract-treated groups when compared with the control ($p<0.05$). These results are presented in Table 3.

Table 1. Showing the amounts of the phytochemical agents identified in *Ajumbise* extract

Phytochemical parameters	Quantities ethanol extract (mg/100 g)	Quantities in chloroform extract (mg/100 g)	Quantities in diethyl ether extract (mg/100 g)	Quantities in aqueous extract (mg/100 g)
Tannins	5.40±0.07	3.93±0.07	3.69±0.05	4.96±0.09
Flavonoids	15.20±0.34	9.78±0.43	10.55±0.56	12.88±0.47
Saponins	9.69±0.10	7.26±0.31	7.18±0.45	11.72±0.33
Steroids	7.48±0.51	8.31±0.58	9.46±0.52	6.39±0.39
Terpenes	4.55±0.05	4.22±0.08	4.05±0.06	4.90±0.08
Alkaloids	19.71±0.22	13.75±0.71	12.49±0.33	17.35±0.59
Glycosides	2.92±0.15	2.73±0.12	2.51±0.23	2.81±0.29
Phenols	12.02±0.32	8.48±0.50	9.54±0.68	11.52±0.32

Results are presented as mean ± standard deviation for n = 3

Table 2. Showing effects of the various solvent extracts on serum levels of PGE2, PGE1, IL-6 and IL-1b in nulliparous rats

Treatment groups	PGE2 (pg./ml)	PGE1 (pg./ml)	IL-6 (pg./ml)	IL-1b (pg./ml)
Control	212.06±3.92^{c}	191.07±3.99^{a}	192.52±3.45^{b}	113.27±2.76^{c}
Ethanol extract (500 mg/kg)	204.53±4.88^{c}	238.10±4.31^{b}	188.71±6.11$^{a, b}$	86.35±2.80^{a}
Chloroform extract (500 mg/kg)	124.00±3.57^{a}	183.33±4.97^{a}	185.39±4.12^{a}	86.15±2.78^{a}
Diethyl ether extract (500 mg/kg)	123.53±4.60^{a}	191.51±5.03^{a}	194.56±3.94^{b}	90.75±1.69^{b}
Aqueous extract (500 mg/kg)	141.63±4.80^{b}	243.58±4.21^{b}	185.32±4.32^{a}	92.66±1.37^{b}

Results are presented as mean ± standard deviation (n = 5), and values with different superscripts are significantly different (P<0.05) from any paired mean with the column. PGE2 = Prostaglandin E2; PGE1 = Prostaglandin E1; IL-6 = interleukin-6; IL-1b = interleukin-1 beta

Table 3. Showing effects of the various solvent extracts on serum levels of PGE2, PGE1, IL-6 and IL-1b parturient rats

Treatment groups	PGE2 (pg/ml)	PGE1 (pg/ml)	IL-6 (pg./ml)	IL-1B (pg/ml)
Control	202.73±3.68^{d}	180.21±3.24^{a}	192.46±3.82^{b}	163.50±3.69^{b}
Ethanol extract (500 mg/kg)	172.91±1.54^{c}	271.36±4.73^{b}	182.65±2.27^{a}	133.13±5.13^{a}
Chloroform extract (500 mg/kg)	130.64±3.34^{a}	268.50±5.75^{b}	190.45±2.12^{b}	134.95±3.19^{a}
Diethyl ether extract (500 mg/kg)	155.38±1.96^{b}	293.41±2.83^{c}	189.02±2.11^{b}	137.81±2.03^{a}
Aqueous extract (500 mg/kg)	171.65±1.31^{c}	300.076±7.65^{c}	181.79±3.97^{a}	137.23±3.94^{a}

Results are presented as mean ± standard deviation (n = 5), and values with different superscripts are significantly different (P<0.05) from any paired mean with the column. PGE2 = Prostaglandin E2; PGE1 = Prostaglandin E1; IL-6 = interleukin-6; IL-1b = interleukin-1 beta

4. DISCUSSION

Inflammation concerns the responses of cells or tissues to injuries caused by internal or external stimuli and explains the underlying molecular mechanisms and complex network of integrated signals between immune cells and the injured tissues [7]. The recruitment and activation of immune cells at the site of inflammation lead to the release of pro-inflammatory cytokines such as IL-6 and IL-1b which are involved in the generation and maintenance of pain [8-12]. The non-treated parturient group showed relatively elevated IL-6 and IL-1B. This is consistent with the report that parturition is initiated and sustained by inflammatory cytokines and other inflammatory agents [13,14]. Findings in this study show that *Ajumbise polyherbal* extract treated nulliparous and parturient rats had significantly reduced serum levels of IL-6 and IL-1b, suggesting that the extract may contain active compounds with anti-inflammatory properties that may inhibit the signalling cascade for secretion of proinflammatory cytokines. The mechanisms may not be fully understood within the scope of this study. What is unknown from this study is the specific active agent (s) in the polyherbal formulation that exhibits the lowering of IL-6 and IL-1B or whether the significant reduction of sera IL-6 and IL-1B are due to the synergistic effect of the formulation. However, the traditional use of *Ajumbise* as an anti-inflammatory formulation used postpartum to manage pains and inflammations may be explained by the finding [15].

The analgesic property of *Ajumbise* polyherbal had earlier been reported [5]. Pain is a common experience during menstruation and parturition due to the increased activity of these pro-inflammatory cytokines among other mediators (Lois, 2003). Dysmenorrhea and menorrhagia, which exaggerate tissue destruction in normal menstruation, are pathologies of menstruation which present with cramps and abnormal uterine bleeding (AUB). Current treatment strategies adopt the use of non-steroidal anti-inflammatory drugs which target the inhibition of cyclo-oxygenase enzymes [16] via a reduction in body levels of pro-inflammatory cytokines [17]. In this study, the extract may have achieved this effect of pro-inflammatory cytokine lowering via the same pathway, which may be the reason for its acclaimed analgesic property and a promising agent for the management of postpartum and menstruation-associated pains [5] to serve as an alternative to other agents which are in current for such health issues [18-20]. Recent scientific findings show that alkaloids, flavonoids and terpenoids are phytochemicals with pain-inhibition activities [21]. The presence of phytochemicals in *Ajumbise* has also been reported [6].

Findings here also showed that aqueous and ethanol extracts of *Ajumbise* increased PGE1 levels in the treated rats (nulliparous and parturient). Prostaglandin E1 (PGE1) has been used in obstetric practice for labour induction and cervical ripening and in the treatment of postpartum haemorrhage (Hofmeyr et al., 2010; Carbone et al., 2013; Conde-Agudelo, et al., 2013; Alfirevic and Aflaifel, 2014). This may be why in recent times, the agent has been used as an abortifacient (Malik and Dua, 2022). The fact that in this study, extracts prepared from polar solvents increased PGE1 levels in the rats suggests that the extract may contain active abortifacient principles, agreeing with Ijioma et al., [2] who reported that some components in *Ajumbise* exhibited uterine contractile effects. The lowering of serum PGE2 concentrations in the non-polar solvent extract-treated rats further buttresses the finding that *Ajumbise* may contain uterotonic agents as already reported. This is because PGE2 increases uterine contractions and increases uterine blood flow to sustain the contractions [22-25]. Saponins have been implicated in uterine contractions and findings in this study have shown evidence that these phytochemicals are significantly present in the extracts of *Ajumbise* polyherbal used.

5. CONCLUSION

Findings from this study have shown that *Ajumbise* polyherbal extract may be a potent anti-inflammatory agent having lowered serum levels of pro-inflammatory cytokines in albino rats. The increase in PGE1 and PGE2 levels in rats treated with the extracts also suggests possible uterotonic effects, agreeing with existing literature data. The inferred anti-inflammatory and possible uterotonic effects are attributable to the rich alkaloids, flavonoids, terpenoids and saponins contents of the different solvent extracts. Further studies with the polyherbal medicament need to uncover the pharmacological merits of the constituting herbal materials or establish the findings of the study as their synergistic effects of the viz-a-viz proinflammatory cytokines and prostaglandins.

6. LIMITATIONS OF THE STUDY

The study is designed to make unbiased observations of the effects of the formulation as applied in traditional use hence the use of all constituting herbal materials. The scope of this study can neither affirm the pharmacological property of constituting herbal materials nor establish the synergistic property of the formulation. It is beyond the scope of the design to explain the sublime mechanism of the observations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were carried out in accordance with international standards for the care and use of laboratory animals as prescribed by the ethical committee of the college of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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