*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 3 Ser. I (May. –June. 2022), PP 01-08*
*www.Iosrjournals.Org*

# Evaluation of Analgesic Activities of Polyherbal Formulation in Wistar Rats.

*C.OAhonsi ; D.SBibinu; S.OEtatuvie; T.O Adebisi and N.BFadok
*Quality Control Unit, Department of Product Development/Quality Assurance,*
*Nigeria Natural Medicine Development Agency Lagos, Nigeria*
*Address for Correspondence: ohikhatemen85@gmail.com. 08063810806*

***Abstract***
*Currently used analgesic agents are opioids & NSAID (non-opioid), all of these are associated with certain adverse effects, research into new potent herbal drug formulations is urgently needed. Theaim of the study was to investigate the analgesic activities of a polyherbal formulation (PHF)Developed by Nigeria Natural Medicine Development Agency (NNMDA) using standard methods. Analgesic activities was evaluatedusingthree experimental models, acetic acid-induced abdominal writhing, Eddy's hot plate method,and Tail flick methods, wistar rats were divided into six groups of five rats in each group. The negative control group received normal saline (5ml/kg), positive control group received Aspirin (100mg/kg) and the other four groups received polyherbal formulation methanolic extract 0.5,1.0 and 1.5 mg/kg respectively.*
*In acetic acid writhes PHF extract at all doses showed significantperipheral activity in a dose dependent manner ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) as compared tothe negative control.In eddy hot plate model all the three test doses of the extract and the standard drug morphine sulphate produced significant central analgesic activity ($p < 0.05$, $p < 0.01$ and $p < 0.001$) by delaying the reaction time at all-time intervals of observation when compared with the negative control. The maximum analgesic activities of all doses of the extract (0.5, 1.0 and 1.5 mg/kg) were observed at 120 mins (176.22±44.55, 256.94±14.47 and 786.65±126.63) as compared with the standard drug (morphine sulphate 10 mg/kg) that produced287.32±48.43 analgesic activity.The tail flick method shows moderated significant reduction with the mice tail flick induced irritancy time, the administration of graded doses of the herbal formulation and 10 mg/kg of Indomethacin as standard significantly increase the tail flick latency after 120 min and 180 mins respectively at a constant infrared light beam maintained, also moderated reduction in motility after the administration of extract indicating its neural depressive effect of the formulation as observed in the study. This explains the sluggish movement and slow response (reduce movement) of the animals after the administration of PHF.Isolation of the Phytoconstituents of the formulation may provide new perspective in drug Development.*

***Keywords:*** *Polyherbal formulation (PHF), Non-steroidal anti-inflammatory drugs (NSAIDs), Analgesic, herbal drug.*

---

Date of Submission: 28-04-2022 Date of Acceptance: 10-05-2022

---

## I. Introduction:

According to international association for the study of pain defines pain as an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage, or described in terms of such damage (Raja et.al., 2020). pain is a sign of tissue lesions due to mechanical, chemical or physical stimulation. The perception of pain is controlled by the neurosensory system and afferent nerve lanes, which are particularly responding to potential damage (Ghosh et.al., 2011). It also stimulates the liberation of some substances that are called pain mediators, such as histamine, bradykinin, leukotriene and prostaglandin (Ghosh et.al., 2011). All of these pain mediators stimulate the pain receptors that channel the stimulation through to the brain via nerve points that have many synapses through spinal cord, marrow advanced, and midbrain. For the treatment of this pain, there is a class of drugs known as analgesics.Analgesics relieve pain, without affecting its cause. Analgesics are divided into two groups, opioid analgesic and non-opioid analgesic (Tripathi 2018). None of the currently used analgesic agents i.e., opioid & nonopioids fulfils the criteria for ideal analgesics. Repeated use of non-steroidal anti-inflammatory drugs (NSAIDs) may induce several adverse effects, such as gastrointestinal lesions or renal and liver failure (Rao and Knaus, 2008). NSAIDs may cause or exacerbate gastrointestinal upsets, peptic ulcers, platelet dysfunction. It may cause bronchospasm resulting in exacerbation of bronchial asthma. Opioids are reserved for severe pain. Adverse effects of opioids include sedation, nausea, vomiting, constipation, physical dependence, tolerance, respiratory depression & urinary retention.During the last few years, a great deal of interest has been given to medicinal

plants as potential therapeutic agents in the treatment of pain and inflammationRegardless of the availability of sufficient drugs, pain and inflammation remain the most challenging and devastating health problems which affect 80% of adult population worldwide (WHO 2012). They are considered as the major clinical, social, and economical problem in most communities around the world (Calati et.al., 2015). Untreated and persistently prolonged pain is the most pervasive disorder that results both physical damage and psychological disorders (Henschke et.al., 2015).Untreated inflammatory disorder may also lead to the progression of serious inflammatory diseases, including asthma, autoimmune disease, chronicinflammation,glomerulonephritis, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, hypersensitivities, hay fever, atherosclerosis, and rheumatoid arthritis. These devastating conditions are the major cause of disabilities and can lead to death unlessthey are properly managed and controlled (Geremew et.al., 2015). The currently available standard drugs for pain and inflammation remain the mainstay for managing and treating these disorders. However, they are associated with many side effects and toxicities, such as gastric irritation, gastric ulcer, alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition which may result in increased bleeding. Using NSAIDs results inincreased risk of cardiovascular effects especially, in patients taking COX-2 inhibitors (Geremew et.al., 2015; Tamrat et.al., 2017). Opioid analgesics are also associated with many unwanted side effects and toxicities, including drowsiness, nausea and vomiting, pruritus, constipation, disturbing hormonal homeostasiss, hearing loss, tolerance, physical dependence, addiction,and respiratory problems.

The use of conventional drugs for the treatment of pain and inflammation has largely resulted in various side effects. These challenges have triggered scientific researchers all over the world in search of alternative therapy (Arome et.al., 2014). Research into new effective and safe analgesic agents with satisfactory tolerability and proven efficacy is urgently needed (Brower et.al., 2000). Hence, analgesic drugs lacking these adverse effects are being searched all over the world as an alternative to NSAIDs and opiates. These have necessitated the investigation of the efficacy on plant-based drugs used in traditional medicine because they are affordable, have little or no side effects. In an extensive literature search we came to know that in many pharmaceutical institutes many animals' experimentation models are used to establish analgesic, anti-inflammatory & antipyretic properties to different medicinal herbs (Sharm et.al.,2010; Kumar and Singh, 2019; Khan andNaz 2018; Adedapo et.al., 2015; Chowdhury et.al., 2015; Mohamed et.al., 2011; Deepika et.al., 2016).The aim of the study is to evaluate the analgesic activities of a poly herbal drug developed by the Nigeria Natural Medicine Development Agency usinganimal experimental models.

## II. Materials And Methods

**Phytochemical Screening:**

The preliminary phytochemical screening of polyherbal formulation was performed by the method described by Gupta et al. (2012). Methanolic extract of the polyherbal formulation (PHF) was used for evaluating the presence of phyto-constituents.

**Acute Toxicity Studies:** Acute toxicity study was performed in accordance with OECD guidelines (2008). No adverse effect or mortality was detected in wistar rats up to 1.5gm/kg, p.o. of polyherbal formulation during 24-72h observation periods. For this period, the rats were continuously observed for any behavioural, neurological or autonomic toxic effect and lethality after 24-72h.

**Analgesic Activity Study:** Analgesic activity study of test drug was done by using acetic acid-induced writhing test, Eddy's hot plate method and Tail flick test using hot water bath.

**Acetic Acid-Induced Writhing in Mice:** This method is useful for the evaluation of peripheral analgesic activity of the drug. All animals fasted overnight. Dose calculation of drug for each animal according to body weight in the respective group was done. One hour before inducing writhes with acetic acid, all animals received their respective drug as per body weight (Yongna et.al., 2005). One hour after dosing, writhing was induced in mice by intraperitoneal injection of 0.6% acetic acid in a dose of 10 ml/kg body weight (Daud et.al., 2006). Numbers of writhes were counted for 10 min beginning from 5th min. after the acetic acid injection. Writhing is a response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension (Tsung-chun et.al., 2007). Percentage inhibition of writhing response in each group was calculated by using the following formula.

$$\text{Percentage inhibition} = \frac{N - Nt}{N} \times 100$$

Where N: Average no. of writhes of the control group

Nt: Average no. of writhes of the test group (or standard group).

After this experiment, all animals were put for a watch out period of 14 days.

**Eddy's Hotplate Method:** This method is useful for evaluating the central analgesic activity of a drug. For this experiment, animals of each group were tested for paw lick or jump response after placing them on eddy's hot

plate, and only those that reacted in the range of 3 to 5 sec (up to 15 sec) were used for the experiment. Those who didn't show a response within 15 sec were removed from the experiment. Time interval from placing animals on the surface of the hot plate to licking of hind paws or jumping is termed as hot plate latency period (Kulkarni, 2014). This is called a basal hot plate latency period. After recording the basal hot plate latency period of each animal in all four groups, selected animals of each group received their respective drug as per body weight (Vogal, 2015). 30 min after dosing, animals of each group were placed on Eddy's hot plate analgesiometer one by one, which was maintained at 55 ± 1 ºC temperature. Hot plate latency period for each animal was noted by using stopwatch at 0, 30, 60, 90 & 120 min. after dosing. The mean hot plate latency period for each group was calculated. Cutoff time was 45 sec. As after dosing response time increases, those who did not react till 45 sec were removed from hot plate to reduce chances of burn.

**Tail flick test using hot water bath:**the method of(Sanchez-Mateo *et al*. (2006) was used in the study. six Groups of five mice each were administered with the herbal extract at different concentration (mg/kg b.w). Indomethacin (5mg/kg b.w) and distilled waterrespectively. Thereafter, the terminal 2 cm of the mice tail were immersed in hot water contained in a 500 ml beaker maintained at 55±1°C. A thermometer was placed inside the water to monitor the temperature. Their responses to thermal pain were taken at 30, 60 and 90 minutes after administration of extract, indomethacin or distilled water.

**Grouping of wistar Rats:** Animals were equally divided into five groups viz. A, B, C, D& E. (each group had six animals)

**Group A:** Normal Untreated rats but induced with 0.1% Acetic acid.

**Group B:** Indomethacin treated rats and induced with 0.1% Acetic acid.

**Group C:** Received 0.5 ml of herbal formulation and induced with 0.1% Acetic acid.

**Group D:** Received 1.0 ml of herbal formulation and induced with 0.1% Acetic acid**.**

**Group E:** Received 1.5 ml of herbal formulation and induced with 0.1% Acetic acid.

## III. Results

Preliminary phytochemical screening for secondary metabolites was carried out to detect the presence or absence of different phytoconstituents from 80% ethanol extract of PHF. The presence of tannins, alkaloids, Flavonoids, glycosides, Anthraquinone, TerpenoidPhenol, Steroid,Phlobatanninsand Coumarins were confirmed through qualitative colour changes of test reagents which gave a clue to the possible mechanisms of analgesic effects of the extract, Acetic acid induced writhing is a sensitive method for screening peripheral analgesic effect of compounds. It causes an increase in concentration of prostagladins (PGE2 and PGF2) in the peritoneal fluid.The hot plate method and tail flick method originally described by Woolfe and Mac Donald (1994) has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics. The nociceptors seem to be sensitized by sensory nerves, the involvement of endogenous substances such as PGs may be minimized in this model.

**Analgesic Activity**

**Acetic acid writhing response in mice**

In this test,PHF extract at all test doses employed (0.5, 1.0, and 1.5mg/kg) showed statistically significantperipheral analgesic activity in a dose dependent manner ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) as compared tothe negative control. All 3 test doses of the extract produced increased inhibition of the numbers of writhing with maximum inhibition observed at maximum dose (1.5 mg/kg) ($p< 0.001$). The highest dose significantly decreased the number of writhing ($p < 0.001$) than the lower dose (0.5 mg/kg) and the middle dose (1.0 mk/kg) ($p < 0.05$). Theextent of reduction of writhing in the different doses of the extract was different, i.e., significantly lower in 0.5 mg/kg ($p < 0.001$) and 1.0 mg/kg ($p < 0.01$) while at 1.5 mg/kg it was comparable to the standard drug (Indomethacin at 10 mg/kg)as presented in (Table 1). The results shows moderated significant increase in the number of abdominal contractions after 0.1% Acetic acid induced writhing effect (licking of abdomen), the administration of graded doses of the herbal formulation and 10 mg/kg of Indomethacin as standard significantly reduced the writhing effect after 60 min, 90 min and 120 min (17.00±1.08 - 9.25±1.49, 12.00±1.08 - 6.00±1.08 and 18.00±2.86 - 8.00±1.47) respectively, the results indicate a promising analgesic property at the high doses to the mice but also moderated reduction in motility was also observe with the mice and a faster (quick) onset of action after the administration indicating its neural sedative (depressive) effect of the formulation as observed in the study.

**Table 1: The Effect of Herbal Formulation on Acetic Acid Induced Writhing Response in Wistar Rat**

| Group | Weight (g) | 0min | 30min | 60min | 90min | 120min | 120min |
|---|---|---|---|---|---|---|---|
| **A** | 23.25±2.87 | 28.00±1.68 | 27.00±2.65 | 23.50±1.44 | 20.25±2.25 | 22.00±4.49 | 0 |
| **B** | 23.50±4.03 | 25.75±3.75 | 20.75±1.44 | 15.25±0.85 | 11.75±0.48 | 7.75±0.48 | 68.18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **C** | 28.00±2.12 | 25.75±2.81 | 20.75±1.65 | 17.00±1.08 | 11.75±1.32 | 9.25±1.49 | 59.09 |
| **D** | 26.25±2.10 | 22.00±2.94 | 19.50±0.65 | 12.00±1.08 | 9.25±0.85 | 6.00±1.08 | 72.73 |
| **E** | 25.25±2.06 | 25.50±2.75 | 23.00±1.47 | 18.00±2.86 | 10.00±0.41 | 8.00±1.47 | 63.64 |

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.

KEY: GROUP A = Normal Untreated rats but induced with 0.1% Acetic acid, GROUP B = Indomethacin treated rats and induced with 0.1% Acetic acid. GROUP C = Received 0.5 ml of herbal formulation and induced with 0.1% Acetic acid, GROUP D = Received 1.0 ml of herbal formulation and induced with 0.1% Acetic acid, GROUP E = Received 1.5 ml of herbal formulation and induced with 0.1% Acetic acid.

**Table 2: Percentage change between time of treatment**

| Group | 0min | 30min | 60min | 90min | 120min |
|---|---|---|---|---|---|
| **A** | 37.43±1.66 | 4.06±1.58 | 6.18±1.30 | -0.71±2.51 | 9.76±4.04 |
| **B** | 22.15±3.36 | 18.93±4.24 | -5.01±8.43 | -16.59±7.00 | -8.11±1.71 |
| **C** | 30.68±4.55 | -6.59±1.11 | 2.24±5.14 | -8.39±5.44 | -13.21±2.60 |
| **D** | 25.89±2.02 | -7.65±1.59 | -3.64±2.10 | -6.62±2.35 | -17.05±1.17 |
| **E** | 19.41±1.43 | -10.72±1.92 | -10.08±1.11 | 1.19±2.83 | -18.87±2.02 |

**Mean ± SEM of percentage value**
**Key = - indicate reduction (decrease) and + indicate increase**
Moderated significant reduction was observed within 2- 4 hrs after treatment with Herbal formulation.

**Eddy's Hotplate Method**

The hot plate method was performed to determine the central analgesic activity of PHF extract in mice. In this model all the three test doses of the extract and the standard drug Indomethacinproduced significant central analgesic activity ($p < 0.05$, $p < 0.01$ and $p < 0.001$) by delaying the reaction time at all-time intervals of observation when compared with the negative control **(Table 2)**. The maximum analgesic activities of all doses of the extract (0.5, 1.0 and 1.5 mg/kg) were observed at 120mins of observation with the respective values of 176.22±44.55, 256.94±14.47, and 786.65±126.63 as compared with the standard drug (Idomethacin 10mg/kg) that produced 287.32±48.43 analgesic activity and it was observed that 0.5mg/kg and 1.0mg/kg concentration of herbal formulation was significantly compared to the standard. The results shows moderated significant effect on mice with hot plate induced pain, the administration of graded doses of the herbal formulation and 10 mg/kg of Indomethacin as standard significantly increase the latency time of the mice after 60min, 90min and 120min respectively, the results shows a promising analgesic potency at the high doses to the mice but also moderated reduction in motility after the administration indicating its neural depressive effect of the formulation as observed in the study.

**Table 3: The effect ofherbal formulation on Hot plate induced pain in Wistar Rat**

| Group | Weight (g) | 0min (Sec) | 30min (Sec) | 60min (Sec) | 90min (Sec) | 120min (Sec) |
|---|---|---|---|---|---|---|
| **A** | 25.00±1.58 | 30.00±2.12 | 33.75±0.75 | 35.75±0.63 | 32.50±2.02 | 27.50±2.02 |
| **B** | 24.25±1.11 | 24.00±2.42 | 54.50±2.02 | 73.00±3.29 | 91.50±2.90 | 89.50±3.93 |
| **C** | 24.00±1.68 | 30.25±4.92 | 50.25±3.99 | 68.75±5.47 | 74.25±6.42 | 77.25±1.93 |
| **D** | 22.50±1.32 | 33.25±2.29 | 48.75±6.60 | 72.00±3.56 | 90.00±2.48 | 118.00±5.96 |
| **E** | 27.75±1.31 | 25.50±5.64 | 62.75±4.80 | 101.25±8.65 | 145.25±14.40 | 205.00±7.63 |

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.
KEY: GROUP A = Normal Untreated rats but hot plate induced pain, GROUP B = Indomethacin treated rats and hot plate induced pain. GROUP C = Received 0.5 ml of herbal formulation and hot plate induced pain, GROUP D = Received 1.0 ml of herbal formulation and hot plate induced pain, GROUP E = Received 1.5 ml of herbal formulation and hot plate induced pain.

**Table 4: Percentage change between time of treatment**

| Group | 0min (%) | 30min (%) | 60min (%) | 90min (%) | 120min (%) |
|---|---|---|---|---|---|
| **A** | 14.20±8.44 | 6.11±3.30 | -8.7893±7.08 | -15.41±3.59 | -7.10±9.46 |
| **B** | 132.64±20.65 | 33.92±2.92 | 25.6070±2.38 | -2.18±3.13 | 287.32±48.43 |
| **C** | 73.61±18.04 | 37.94±9.47 | 7.8937±1.55 | 6.60±9.91 | 176.22±44.55 |
| **D** | 45.31±15.17 | 61.22±33.94 | 25.8476±6.44 | 30.93±3.59 | 256.94±14.47 |
| **E** | 167.15±33.47 | 61.77±7.65 | 42.99±4.34 | 44.02±10.02 | 786.65±126.63 |

**Mean ± SEM of percentage value**
**Key = - indicate reduction (decrease) and + indicate increase**
Moderated significant elevation was observed with the 2 hrs to 4$^{th}$ hrs after treatment with Herbal formulation.

**Tail flick induced pain in Wistar Rat:**

In tail flick method, results showed moderated significant reduction with the mice tail flick induced irritancy time, the administration of graded doses of the herbal formulation and 10mg/kg of Indomethacin as standard, significantly increase the tail flick latency after 90 min and 120min respectively at a constant infrared light beam maintained. The maximum analgesic activities of all doses of the extract (0.5, 1.0 and 1.5 mg/kg) were observed at 120min of observation with the respective values of159.79±68.47, 224.61±25.27, 282.75±52.86, compared with the standard drug (Idomethacin 10mg/kg) that produced 124.65±29.61 analgesic activity. the results shows a promising analgesic property at the high dose to the mice but also moderated reduction in motility after the administration indicating its neural depressive effect of the formulation as observed in the study. This just justify the sluggish movement and slow response (reduce movement) of the animals after the administration.

**Table 5: The effect ofherbal formulation on Tail flick induced pain in Wistar Rat**

| Groups | Weight(g) | Initial | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|---|
| **A** | 20.25±2.66 | 47.25±11.61 | 36.25±3.22 | 21.50±1.32 | 44.75±2.66 | 42.00±5.35 |
| **B** | 28.00±1.68 | 45.25±6.61 | 59.25±2.90 | 79.75±8.87 | 69.00±6.72 | 96.50±4.03 |
| **C** | 22.75±1.49 | 45.25±8.57 | 61.25±7.98 | 69.75±3.71 | 67.25±4.55 | 100.50±4.35 |
| **D** | 27.00±2.27 | 41.75±4.66 | 63.75±2.46 | 73.50±3.57 | 86.75±3.90 | 132.00±4.02 |
| **E** | 24.25±1.25 | 52.50±6.20 | 86.75±5.92 | 101.25±8.44 | 140.00±12.38 | 191.25±5.47 |

**Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.**
KEY: GROUP A = Normal Untreated rats but tail flick induced irritancy, GROUP B = Indomethacin treated rats and tail flick induced irritancy. GROUP C = Received 0.5 ml of herbal formulation and tail flick induced irritancy, GROUP D = Received 1.0 ml of herbal formulation and tail flick induced irritancy, GROUP E = Received 1.5 ml of herbal formulation and tail flick induced irritancy.

**Percentage change between time of treatment**

| Groups | Initial (%) | 30min (%) | 60min (%) | 90min (%) | 120min (%) |
|---|---|---|---|---|---|
| **A** | -9.67±18.81 | -38.40±8.64 | 110.48±18.16 | -3.13±17.73 | 10.00±33.40 |
| **B** | 36.91±15.42 | 33.94±12.11 | -10.46±11.48 | 42.43±10.39 | 124.65±29.61 |
| **C** | 45.27±20.74 | 19.77±16.87 | -3.65±3.30 | 50.31±5.28 | 159.79±68.47 |
| **D** | 58.31±19.03 | 16.08±8.61 | 18.16±2.53 | 52.72±5.69 | 224.61±25.27 |
| **E** | 69.11±13.02 | 16.41±2.65 | 40.00±14.11 | 39.63±11.89 | 282.75±52.86 |

**Key = - indicate reduction (decrease) and + indicate increase**
Moderated significant elevation was observed with the 60 - 120mins after treatment with Herbal formulation.

## IV. Discussion

**Preliminary phytochemical screening:** The preliminary phytochemical screening of polyherbal formulation (PHF) has shown the presence of tannins, alkaloids, Flavonoids, glycosides, Anthraquinone, Terpenoid Phenol, Steroid, Phlobatannins and Coumarins. The effects observed with PHF could possibly be due to the synergistic

actions of these compounds. This findings were similar to Previous phytochemical screening on a polyherbal drug which revealed the presence of alkaloids, polyphenols, saponins, phytosterols, carotenoids, lignans, sesquiterpene alcohols, acetylenic and thiophene compounds, terpenoids, and essential oil (Hymete et.al., 2007). It is proved that the polyherbal formulation (PHF) contained secondary metabolites including saponin, tannin, alkaloids, phenols, flavonoids, glycosides and steroids as identified by phytochemical screening in the present study. So, it can be said that the analgesic effects shown by the extract may be due to the presence of these aforementioned and currently identified phytoconstituents. This suggestion is in line with the report by Tamrat et al. (2017) that, phytoconstituents like, alkaloids, flavonoids, steroids, and tannin isolated from medicinal plants possess a significant analgesic activity (Debebe et.al., 2007; Tamrat et.al., 2017).

Acetic acid-induced writhing test was selected to detect the peripheral analgesic activity of the extract, due to its sensitivity and ability to detect antinociceptive effects of natural products and test compounds at dose levels which remains inactive for other methods, acetic acid-induced writhing test is a well recommended model for screening the peripheral analgesic potentials of test compounds (Subedi et.al., 2016). Intraperitoneal injection of acetic acid causes irritation and stimulation of the peritoneal cavity that triggers the synthesis and release of various endogenous inflammatory mediators such as histamine, serotonin, bradykinin substance P, and PGs (Konaté et.al., 2012). These various endogenous inflammatory mediators elicited chemical-induced visceral pain which is characterized by constriction of abdominal muscles together with the extension of the forelimbs and elongation of the body. That is why the acetic acid-induced writhing test is considered as a model of visceral pain (Tadiwos et.al., 2017). This model has also been associated with increased level of PGE and PGF2a. Increasing PG levels within the peritoneal cavity enhances inflammatory pain by increasing capillary permeability and activating primary afferent nociceptors (Demsie et.al., 2019). PHF extract at all doses employed (0.5, 1.0 and 1.5mg/kg) significantly ($p < 0.01$, and $p < 0.001$) showed peripheral analgesic activities by reducing the number of writhing with the respective values of 59.09%, 63.64%, and72.73%, as compared with the control 68.18%. These findings confirmed that the peripheral analgesic activity of the extract increased from the lower dose (0.5 mg/kg) to the higher dose (1.5g/kg) in dose dependent manner. The increase in analgesic activity with increasing doses of the extract might be attributed with an increase in concentration of phytoconstituents that possess analgesic activity with the maximum dose. The possible mechanism by which the extract produced peripheral analgesia in this model might be associated with inhibiting the synthesis and release of various endogenous inflammatory/pain mediators and suppression of the sensitivity of peripheral nociceptors in the peritoneal free nerve endings for chemical-induced pain. These proposed mechanisms are in line with the principles that stated, any agent that decreases the number of writhing will demonstrate analgesia by inhibiting the synthesis and release of prostaglandin (PGs), and by inhibiting the peripheral pain transmission (Tadiwos et.al., 2017; Debebe et.al., 2007). The second model used was the Eddy hot plate test, in which the supra-spinal nociception and the involvement of central antinociceptive mechanism were detected (Debebe et.al., 2007). Since the paws of wistar rats are very sensitive to heat at temperatures which are not damaging the skin, the central antinociceptive mechanisms of the extract were detected by introducing the mice to the constantly heated plate and by observing the reaction times, namely jumping, withdrawal, and licking of the paws. The time until these responses occurred was prolonged after administration of centrally acting analgesics (Sun et.al., 2018). This model was selected to evaluate the central analgesic potential of the extract because of its sensitivity to strong analgesics, limited tissue damage with a cut-off time of 15 sec, which is usually applied to limit the amount of time the mouse spends on the hot plate. The model also requires less time and measurements are usually accurate. The extract at all test doses (0.5 mg/kg, 1.0 mg/kg and 1.5 mg/kg) significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$ respectively) reduce the pain threshold by increasing the reaction time starting from 30 min of observation onwards as compared to the negative control. The maximum analgesic effects of the extract were observed at120 min of observation time with their respective values of 159.79±68.47, 256.94±14.47 and 786.65±126.63as compared with the standard drug (idomethacin10 mg/kg) which showed a percentage analgesic value of 287.32±48.43 at this time. At all times of observation, the doses of the extract showed analgesic activities in dose dependent manner. The doses of the extract took longer time (peak time) to attain for maximal effect, which for all doses was 120 min. This delay maybe due to a probable time lag for the drug to enter in to the central compartment and distribute into the target site or formation of active metabolites that are endowed with analgesic activity with better half-life. A relatively better action of 1.5mg/kg at all observation time may be attributed by the presence of high concentration of active metabolites. The possibly proposed mechanism of central analgesic effects of the extract may be by activating the periaqueductal gray matter (PAG) to release endogenous peptides (i.e., endorphin or enkephalin). These endogenous peptides descend the spinal cord and function as inhibitors of the pain impulse transmission at the synapse in the dorsal horn or via peripheral mechanisms involved in the inhibition of PG, leukotriene, and other endogenous substances that are key players in central pain transmission (Badole et.al., 2011).

The tail flick methodin the present study demonstrated a significant ($P<0.01$) analgesic activity. Aspirin produced a significant ($P<0.01$) increase in the reaction time at 0, 30, 60, 90 and 120min following administration as compared with control. The treatment with polyherbal formulation (1.0mg/kg and 1.5mg/kg)

produced dose dependent increase in the reaction time of rats at 60 min and 120min, showing an analgesic effect when compared with control animals. PHF extract at 1.0 and 1.5 mg/kg (159.79±68.47 and 282.75±52.86) significantly elevated the mean basal reaction time as compared to control group at 120mins. The highest nociceptive inhibition was exhibited by PHF (1.5 mg/kg) at 120 min.

The evaluation of analgesic activity through hot plate method was selected in the present study because it has several advantages such as sensitivity to strong antinociceptive and limited tissue damage (Sumitra et.al 2001). Woolfe and MacDonald described this method to be suitable for the evaluation of centrally but not peripherally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PG's may be minimized in this model (Woolfe and MacDonald 1994). Aspirin is responsible to inhibit cyclooxygenase in peripheral tissues by interfering with the mechanism of transduction in primary afferent nociceptors (Rao et.al., 2003). In the hot plate model, the paws of rats are very sensitive to temperatures at 50-55±1ºC (Franzotti et.al., 2000). Increase in pain reaction time (PRT) or latency period indicates a significant analgesic effect of the polyherbal formulation methanolic extract (Ranadran and Basinath, 1986).

## V. Conclusion:

The polyherbal formulation extract possessed peripheral analgesic activity and central pain inhibition potential. Their activity may be attributed to the synergistic effect due to the combination of multiple medicinal plants materials in the formulation. Further analysis on the formulation may reveal the exact nature and structure of the chemical constituents responsible for its analgesic activity, isolation of the Phytoconstituents of the formulation may provide new perspective in drug discovery.

**CONFLICTS OF INTEREST:** The authors declare no conflict of interest

**ACKNOWLEDGEMENT:** The authors are thankful to the Management of Nigeria Natural Medicine Development Agency and Staffs of Emma-Maria Scientific Research Laboratories Delta State Nigeria

## References

[1]. Adedapo AA, Falayi OO and Oyagbemi AA (2015): Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera insome laboratory animals. *Journal of Basic Clinical Physiology and Pharmacology*, 26 (5), pp. 491-499.

[2]. Arome D, Sunday AI, Onalike EI and Amarachi A (2014): Pain and inflammation: management by conventional and herbal therapy. *Indian Journal of Pain*, 28(1), pp. 5-12.

[3]. Bachlav RS, Gulecha VS, Upasani CD (2009). Analgesic and Anti-inflammatory Activity of Argyreia Speciosa roots. *Indian Journal of Pharmacology* 41(4), pp. 158–161.

[4]. Badole SL, Zanwar AA, Ghule AE, Ghosh P, Bodhankar SL. Analgesic and anti-inflammatory activity of alcoholic extract of stem bark of Pongamia pinnata (L.) Pierre. Biomed Aging Pathol. 2012;2(1):19–23. doi:10.1016/j.biomag.2011.11.001.

[5]. Brower V (2000): New paths to pain relief. Nat Biotechnol 18: 387-91.

[6]. Calati RBC, Artero S, Ilgen M, Courtet P (2015). The impact of physical pain on suicidal thoughts and behaviors meta-analyses. *Journal of Psychiatric Research*.71:16–32.

[7]. Chowdhury B, Bhattamishra SK andDas MC (2015) A comparative study on central analgesic activities of ethanol & aqueous extracts of Glycyrrhiza Glabra root in albino rats Pharmacologyonline 3: 660-67.

[8]. Collier HO, Dinneen LC, Johnson CA, Schneider C. (1968) The abdominal constriction response and its suppression by analgesic drugs in mouse. *British Journal Pharmacology* 32, 295-310.

[9]. Daud A, Habib N and Riera S: A Anti-inflammatory, antinociceptive and antipyretic effects of extracts of Phrygilanthus acutifolius flowers. J Ethnopharmacol 2006; 108: 198-03.

[10]. Daud A, Habib N and Riera S: A (2006) Anti-inflammatory, antinociceptive and antipyretic effects of extracts of *Phrygilanthus acutifolius* flowers. *Journal of Ethnopharmacology,*20 (3) pp. 108- 198.

[11]. Tsung-chun L, Yu-zen K, Hsin-wei H, Ying-chun H, Ying-chin L and Wen-huangpeng (2007) Analgesic and antiinflammatory activities of aqueous extract from glycine *tomentella* root in mice. *Journal of Ethnopharmacology* 21 (8), pp.113: 142. .

[12]. Debebe E, Makonnen E, Debella A (2007). Anti-nociceptive effect of the methanolic extract of roots of andrachneaspera in three models of Nociception. Pharmacol Online. (1), pp.41–48.

[13]. Deepika PL, Jagdish, Priyambada S and Sowmya (2016) Analgesic, anti-inflammatory activity of Tinospora Cordifolia (Guduchi) & Valeriana Wallichi (Tagar) in albino rats. IOSR *Journal of pharmacy & biological sciences* (IOSR-JPBS. 11(2): 2319-76.

[14]. Demsie DG, Yimer EM, Berhe AH, Altaye BM, Berhe DF (2019). Antinociceptive and anti-inflammatory activities of crude root extract and solvent fractions of Cucumis ficifolius in mice model. *Journal Pain Research*. 12:1399. doi:10.2147/JPR.S193029.

[15]. Franzotti EM, Santos CVF, Rodrigues HMS, Mourao RHV, Andrade MR, et al.(2000)Anti-inflammatory, analgesic and acute toxicity of Sida cordiforlia L. *Journal of Ethnopharmacology* 72: 273–277.

[16]. Geremew H, Shibeshi W, Tamiru W, Engdawork E (2015) Experimental evaluation of analgesic and anti-inflammatory activity of 80% methanolic leaf extract of Moringa stenopetala Bak. F. in mice. *Ethiopian Pharmacology Journal*. 31(1), pp. 15–26.

[17]. Ghosh, AK, Banerjee M, Mandal TK, Mishra A, Bhowmik M.K (2011). A Study on Analgesic Efficacy and Adverse Effects of Aloe vera in Wistar Rats. Pharmacologyonline. 1: 1098-1108.

[18]. Henschke N, Kamper SJ, Maher CG, (2015) editors. The epidemiology and economic consequences of pain. Mayo Clinic Proceedings. Elsevier;.

[19]. Hymete A, Rohloff J, Iversen TH, Kjøsen H (2007) Volatile constituents of the roots of Echinops kebericho Mesfin. Flav Frag J. 22 (1):35–38. doi:10.1002/ffj.1746

[20]. Khan A andNaz S (2018). Bioactive chromone constituents from Vitex nigundo alleviate pain and inflammation. *Journal of Pain Research* 11: 95-102.

[21]. Kulkarni SK (2014;). Hand book of experimental pharmacology. 3 rd Edn. Vallabh Prakashan, Delhi, publicaion 117.

[22]. Kumar R and Singh S (2019). Effect of Boswellia serrata extract on acute inflammatory parameters and tumor necrosis factor-α in complete Freund's adjuvant-induced animal model of rheumatoid arthritis IJABMR 9(2), pp. 100-06

[23]. Mohamed SF, Atlee WC and Khannan S (2011). Assessment of analgesic, anti-pyretic & anti-inflammatory activity of hydro-alcoholic fraction of Hemidesmous indicus root in experimental animals. Scholars Research Library Der Pharmacia Lettre 3(1), pp. 442-70.

[24]. Nalamachu S (2013) An Overview of Pain Management: The Clinical Efficacy and Value of Treatment. American Journal Manag Care19. 16: 261-266

[25]. OECD (2008), Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071049-en.

[26]. Oh YC, Jeong YH, Cho WK, Ha JH, Gu MJ, Ma JY (2015) Ant-inflammatory and analgesic effects of pyeongwisan on LPS-stimulated murine macrophages and mouse models of acetic acid-induced writhing response and xylene-induced ear edema, *International Journal of Molecular.Science*. 16(1), pp. 1232-1251.

[27]. Onasanwo SA, Elegbe RA. (2006) Antinociceptive and Anti-inflammatory Properties of the leaf Extract of Hedranthera barteri in Rats and Mice. *African Journal of Biomedical Research*. 6(2), pp.109–118.

[28]. Priyambada S, Jagdeesh P and Mayuri T (2015): Dos dependent anti-inflammatory effect of Valeriana wallichii in 0.1ml of 1% carrageenana induced hind paw edema in male albino rats. IOSR-JPBS 10(2), pp. 06-09.

[29]. Raja SN, CarrDB, CohenM, FinnerupNB, FlorH, GibsonS KeefeFJ MogilJS RingkampM, SlukaKA SongXJ, StevensB SullivanMD,TutelmanPR, UshidaT,VaderK. (2020) the reversed international association for the study of pain definition of pain: concept, challenges and compromises. Pain. 1;161(9), pp.1976-1982.

[30]. Ranadran K, Basinath L. A critical Analysis of the Experimental Evaluation of Nociceptive reactions in Animals. Pharm Research. 1986; 3:253–270.

[31]. Rao C.V., Kartik R., Ojha S.K. and Amresh Rao G.M.M., Antiinflammatory and ntinociceptive activity of stem juice powder of Tinospora cordifolia Miers. in experimental animals, Hamdard Medicus. 2005, XLVIII, 102–106.

[32]. Rao C.V., Ojha S.K., Amresh G., Mehrotra S. and Pushpangadan P., Analgesic, antiinflammatory and antiulcerogenic activity of unripe fruits of Aegle marmelos, Acta Pharmaceutica. Turcica., 2003, 45, 85–91.

[33]. Schug SA, Garrett WR,Gillespie G. Opioid and non-opioid analgesics. Best Pract Res Clin Anaesthesiol. 2003;17(1):91–110. doi:10.1053/bean.2003.0267

[34]. Sharma A, Bhatia S and Kharya MD: Anti-inflammatory & analgesic activity of different fractions of Boswellia serrata. Int Journal of Phytomedicine 2010; 2: 94-99.

[35]. Sun K, Song X, Jia R, et al. Evaluation of analgesic and anti-inflammatory activities of water extract of galla chinensis in vivo models. Evid Based Complement Altern Med. 2018;2018. doi:10.1155/2018/6784032.

[36]. Tadiwos Y, Nedi T, Engidawork E. Analgesic and anti-inflammatory activities of 80% methanol root extract of Jasminum abyssinicum Hochst. ex. Dc. (Oleaceae) in mice. J Ethnopharmacol. 2017;202:281–289. doi:10.1016/j.jep.2017.02.036

[37]. Tamrat Y, Nedi T, Assefa S, Teklehaymanot T, Shibeshi W (2017). Antiinflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak. (Moringaceae) in mice models. BMC Complement Altern Med. 17(1), 473-480.

[38]. Tripathi KD (2018) Essentials of medical pharmacology. 8th ed. Delhi: Jaype Brothers Medical Publishers (P) Ltd; . 455.

[39]. Tsung-chun L, Yu-zen K, Hsin-wei H, Ying-chun H, Ying-chin L and Wen-huangpeng (2007) : Analgesic and anti-inflammatory activities of aqueous extract from glycine tomentella root in mice. *Journal of Ethnopharmacology* 113: 142-8

[40]. Vogal HG (2015) Drug discovery and evaluation. In: analgesic, anti-inflammatory and antipyretic activity. 4th ed. New York: Springer, 696.

[41]. WHO Guidelines on the Pharmacological Treatment of Persisting Pain in Children with Medical Illnesses. Thieme New York: World Health Organization; 2012.

[42]. Witkin L.B., Huebner C.F., Galdi F., O'Keefe E., Spitaletta P. and Plummer A (1961) Journal of Pharmacognosy of 2 amino-indane hydrochloride (Su 8629): a potent non-narcotic analgesic, J. Pharmacol. Exp. Ther., , 133, 400–408.

[43]. Woolfe G, MacDonald AD (1994) The evaluation of the analgesic action of pethidine hydrochloride. *J.Pharmacol Exp Ther.* 80:300.

[44]. Yongna Z, Wantana R, Pisit B, Li Z and Rongping Z (2005):Analgesic and antipyretic activities of the aqueous extract of Urtica macrorrhiza in experimental animals. Fitoterapia 76: 91-5


C.O Ahonsi, et. al. "Evaluation of Analgesic Activity of Polyherbal Formulation in Wistar Rats." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(3), (2022): pp. 01-08.