TECHNICAL REPORT

ON

student industrial work experience scheme (SIWES 2)

UNDERTAKEN AT

NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT, IDU
ABUJA, NIGERIA.

BY

SADIQ FADILAT ABDULMUMIN

16/MHS07/030

A STUDENT INDUSTRIAL WORK EXPERIENCE SCHEME REPORT SUBMITTED

TO
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

COLLEGE OF MEDICINE AND HEALTH SCIENCES

AFE BABALOLA UNIVERSITY, ADO-EKITI
EKITI STATE-NIGERIA

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.SC) IN PHARMACOLOGY.
APRIL2020

CERTIFICATION

This is to certify that this work was undertaken by SADIQ FADILAT ABDULMUMIN at NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT, IDU ABUJA, and supervised by Pastor Oni John, with the report prepared and presented to the department of pharmacology and therapeutics, Afe Babalola University, Ado Ekiti (ABUAD),Ekiti state, Nigeria during the 2018/2019 student industrial work experience scheme(SIWES 2).

DEDICATION

This report is dedicated to Almighty Allah, for his mercies and blessing shown on me before, during and after my siwes program. I would also like to dedicate this to my family, especially my mother, my friends, Department of Pharmacology and Therapeutics and to my fellow students that worked with me during the period of this attachment at national institute for pharmaceutical research and development, idu Abuja.

SADIQ FADILAT ADBULMUMIN

300 LEVEL

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

ACKNOWLEDGEMENT

Thanks be to Allah for his blessing, guidance, protection, the courage and opportunity given to me to the successful completion of my SIWES program.

I wish to express my thanks to my beloved parents for their moral and support towards the completion of this program.

My last acknowledgement goes to my supervisor Pastor Oni John, and also my supervisor at NIPRD DR MRS IFEOMA for their relevant suggestion and contribution towards the completion of this program and I also thank the staff and management of NIPRD for their understanding and the opportunity given to me to work in their organization.

My profound gratitude goes to the college of Medicine and Health Sciences, the Provost of the College of Medicine and Health Sciences: Prof. Sanya J. O and the Head of Department, Pharmacology and Therapeutics: Dr. Adeoluwa Olusegun and I also want to specially thank members of the academic and non-academic staff, Afe Babalola University (ABUAD).

TABLE OF CONTENTS

ABSTRACT

The student industrial scheme (SIWES) is a scheme designed but the federal government to expose 200/300 level students in tertiary institutions more to acquisition of practical skills relevant to their course of study for exposure and more experience purpose. The 2018/2019 SIWES was undertaken at NIPRD abuja, I was accepted into their industrial attachment program available for students in tertiary institutions, and I was deployed to the department of pharmacology, there we carried out different studies on plants and laboratory animals.

CHAPTER ONE

INTRODUCTION: BRIEF HISTORY OF SIWES

The student industrial training is the training programme which forms part of the academic standards in various degree programmes for all Nigeria tertiary institutions. It seeks to bridge the gap existing between technology and other professional education programmes in Nigerian tertiary institutions.

The early phase of science and technology in Nigeria was characterized by the theoretical lectures in polytechnics and universities which have proven to be an ill method of teaching. Students in Universities and Polytechnics graduate with little or no technical experience in their course of study.
In the same vein, students' inability to contribute to the society is hampering the growth and development of our country. It was in this view that SIWES was introduced to the Industrial and Educational sector.
SIWES is an acronym for Student's Industrial Work Experience Scheme.
SIWES was established in the year 1973 in order to improve the standard of education in Nigeria in order to achieve the needed technological advancement.

PURPOSE OF SIWES

The objective of student industrial work experience scheme (SIWES) is to enable every student who passed through university or other institution to acquire a practical knowledge of what he/she has learned. Therefore, it is compulsory for every student to satisfy the requirement in his/her academic pursuit.

AIMS AND OBJECTIVES OF SIWES

1. To provide students with industrial skills and needed experience while the course of study.
2. To create conditions and circumstances, which can be as close as possible to the actual workflow.
3. To prepare specialists who will be ready for any working situations immediately after graduation.
4. To teach students the techniques and methods of working with facilities and equipment that may not be available within the walls of an educational institution.
5. To give students the ability to try and apply the given knowledge.

The objectives of SIWES programme are all about strengthening future employees. Such program is successful attempt to help students to understand the underlying principles of their future work. After passing the programs, the student can concentrate on the really necessary factors of his or her work.

CHAPTER TWO

Pharmacological Evaluation of Two Medicinal Plants Used in Treating Arthritis in Northern Nigeria with Focus on *Terminalia avicennioides*

1. Introduction

Arthritis is the inflammation of one or more joints which could be sudden or gradual (Athanasiou et al., 2013). There are over a hundred type of arthritis, but the main types are osteoarthritis and rheumatoid arthritis i.e. rheumatism (March et al., 2014). It could be an autoimmune condition or due to aging. Arthritis is characterized by pain, swelling or tenderness at the joint, loss of function or deformity. Although there is no cure for arthritis, certain drugs (pain relievers), minerals and vitamins, exercise and lifestyle modification are used to manage arthritis.

Over the years, phytomedicines have been used as sources of medicines either as crude or finished pharmaceutical products. Plants have served as useful sources of therapeutic agents such as: quinine from *Cinchona spp* (Willcox, 2004), artemisinin from *Artemisia annua* (Arsenault et al., 2008) and morphine from *Papaver somniferum* (Rokia, 2011) among others. Due to the longstanding use of medicinal plants, they are believed to be quite safe, cheap and may be better alternatives to conventional drugs. These plants have been identified with activities ranging from antimicrobial, anti-plasmodial, analgesic, anti-inflammatory, antihelminthic, antidiabetic, antiviral to sleep-inducing, ulcer preventing and ameliorating of respiratory conditions.

Cassia sieberiana, a shrub or tree of the family Fabaceae and *Terminalia avicennioides* a shrub of the family Combretaceae have been reported to have several ethnomedicinal uses among which is the management of arthritis (Eatokun et al., 2017, Salihu et al., 2018) but from the literature review done on these plants, there are no previous studies published on their effects on pain and inflammation. This study therefore aims to evaluate the effects of *Cassia sieberiana* root and *Terminalia avicennioides* stem bark extracts on pain and inflammation with focus on *Terminalia avicennioides.*

2. Materials and Methods

2.1 Plant materials

The root of *Cassia sieberiana* and the stem bark of *Terminalia avicennioides* were collected from Chaza in Suleja, Nigeria. Botanical samples were identified at the herbarium of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for pharmaceutical research & Development (NIPRD) where the voucher specimens (NIPRD/H/6981 and NIPRD/H/6982) was deposited.

Immediately after collection, the plant materials were washed clean with water, cut to smaller pieces, dried and pulverized. Weighed quantities of each of the pulverized plant materials were soaked with 70% ethanol and left to macerate for 48 h with intermittent agitation. The aqueous extracts were also be prepared. Resulting extracts were passed through a clean muslin cloth, then through a filter paper. The filtrates were concentrated using a water bath at 55^{o}C and then refrigerated prior to use.

2.2 Animals

Swiss albino mice and rats of both sexes sourced from the Animal Facility Centre of the Department of Pharmacology and Toxicology, NIPRD were used. The animals were

maintained under standard environmental condition with free access to standard diet and water. Six groups of six (6) animals were used for each model. Four of the groups were the treatment, one standard and one control.

2.3 Determination of phenolic content

2.3.1 Preparation of gallic acid calibration plot

A 0.1 mg/ml stock solution of gallic acid was prepared by weighing 1 mg of gallic acid and dissolving with distilled water. Serial dilutions of the stock solution (0.0015625 – 0.1 mg/ml) were prepared and treated as follows: A 2.5 ml of 10% Folin Denis reagent was added to 1 ml of gallic acid solution. The mixture was allowed to stand for two minutes at room temperature after which 2 ml of sodium carbonate solution (75 g/L) was added. The resulting mixture was maintained at 50^{o}C for 15 min in a water bath and afterwards, cooled in ice – cold water for 3 min. Then absorbance was read spectrophotometrically at 760 nm.

2.3.2 Phenolic content assay of extract

Solutions of the extracts (1 mg/ml) were prepared in distilled water. Then 1 ml of each extract were transferred into a test tube and treated as described for gallic acid. The results were obtained in duplicates. Concentration of phenolics were determined from the standard gallic acid plot and result obtained were expressed as gallic acid equivalent per gram of extract.

The radical scavenging activity will be expressed as IC50 by extrapolation at 50% from the plot of percentage inhibition of the extract against concentration.

2.4 Acute toxicity test

An acute oral toxicity study was performed according to the Organization for Economic Cooperation and Development guidelines 423 on limit toxicity testing (OECD, 2008). The study animals (n=16) were divided into four groups of four mice per cage with one mouse in each group serving as the control. The animals were fasted 24 h with access to drinking water prior to the test. Each group received 2000 mg/kg of either the ethanol or aqueous extract of *Cassia sieberiana, Terminalia avicennioides* or water (10 ml/kg). Then, the animals were continuously observed during the first 1 h, periodically observed during the first 24 h, with particular attention during the first 4 h, and observed daily thereafter. The animals were then observed for 14 days for mortality and other signs of toxicity manifestation (OECD, 2008).

2.5 Formalin-induced paw edema in rats

The anti-inflammatory activity was evaluated in six groups of six (6) animals randomized based on their paw volume. The extracts at the dose of 200 mg/kg body weight was administered orally 30 min before the subcutaneous injection of 0.1 ml of 2.5% formalin into the sub plantar tissue of the right hind paw of the rat. The control group received 10 ml/kg water while the reference drug indomethacin 10 mg/kg was given to standard group 30 min prior to induction of edema. Paw sizes were measured with a plethysmometer 30 min before administration of formalin, then thereafter at 30 min, 1 h, 2 h and 24 h after the injection of the inflammatory agent. The individual records were used to determine the average size for each group (St) and then the percentages of variation or percentages of edema by comparison with the average size obtained for each group before any treatment (So). Percentages of inhibition were obtained for each group and at each record using the following ratio:

[(St – So) control – (St – So) treated] x 100/ (St – So) control.

Where “St” is the mean paw size for each group after formalin treatment and “So” is the mean paw size obtained for each group before formalin treatment.

2.6 Acetic acid-induced writhing

This test was conducted as per the method described by Koster et al. (1959). 36 Swiss mice were randomly divided into six groups of, six mice per group. Group I was treated with water 10 ml/kg and served as control; group II with Aspirin (150mg/kg); groups III to VI received aqueous solution of ethanol and aqueous extracts of *Cassia sieberiana* and *Terminalia avicennioides* (200 mg/kg. All treatments were administered p.o. 1 h after treatment, each mouse was administered with 10ml/kg of 0.6% acetic acid (i.p.) and the number of writhing displayed by each mouse was counted and recorded for 15 min.

2.7 Chronic inflammation in mice

2.7.1 Administration of drugs

The animals were allocated to six groups with 8 mice per group: formalin control, normal saline control, 900, 300 and 100 mg/kg with the TAE administered groups, 5 mg/kg of indomethacin (Sigma, MO, USA)-treated groups. Three different dosages of TAE were selected based on the results on the acute inflammatory studies, and 5 mg/kg of intraperitoneal treatment of indomethacin was also selected from previous report (Mirshafiey et al., 2005). TAE aqueous and ethanolic extracts were orally administered once a day for 10 days, while the indomethacin dissolved in saline were intraperitoneally administered at a volume of 10 ml/kg. In the control group, distilled water was orally administered instead of TAE as same methods.

2.7.2 Induction of chronic inflammation

One hour before TAE administration or indomethacin injection, a subaponeurotic injection of 0.02 ml of 3.75% formalin (Sigma, MO, USA) was administered to the right hind paw (Planta pedis) on the first and third days of the experiment. In the case of the control, the same volume of saline and formalin as that used in the other dosing groups, was administered in the same region using the same method as described previously (Kim et al., 2010).

Daily body weights of all experimental animals used in this study were measured from 1 day before the start of the experimental period to 10 days of treatment.

2.7.3 Measurement of Paw size

The volumes of the right hind paws were measured using a Plethysmometer (Ugo Basile, Italy) and recorded once a day for 10 days at 1 h before the first formalin injection, at 1 h before the second formalin injection or 2 h before TAE treatment.

At sacrifice, the wet-weight of the right hind paws was measured, and the relative weight (%) was calculated using the body weight at sacrifice and absolute weight: Relative paw weight (% of body weight) = (Absolute weight/Body weight at sacrifice) × 100.

2.7.4 Measurement of nitrite from paw homogenate

Samples from plantar paw skin were collected after the study, homogenized in 2000 µL of saline, and nitrite concentration was determined by the Griess reaction as an indicator of nitric oxide production. After centrifugation at 10,000 ×g and 4 ^{o}C, 100 µL of the homogenate was incubated with 100 µL of Griess reagent for 5 min at 25^{o}C, and nitrite concentration was determined by measuring the optical density at 550 nm (Insert equipment) in reference to a standard curve of $NaNO_2$ solution. Results are expressed as µmol of nitrite per mg of tissue.

2.8 Statistical Analysis

All data obtained were analyzed using GraphPad Prism 6.00 for Windows. Results were analyzed by one-way analysis of variance (ANOVA) and further subjected to Dunnett’s *post hoc* test for multiple comparisons. Differences between means was accepted as significant at $P < 0.05$.

CHAPTER THREE

3.0 Results

3.1 Phenolic content assay

With reference to the gallic acid calibration plot, the concentrations of phenolics in each extract is as follows:

Table 1: Mean absorbance and estimated gallic acid equivalent (GAE) of extracts

Sample (1 mg/ml)	Mean absorbance (nm)	Concentration of Phenolics (GAE)
Eth. T.A	2.009 ± 0.000	0.136
Aq. T.A	1.999 ± 0.003	0.135
Eth. C.S	1.813 ± 0.243	0.122
Aq. C.S	0.8315 ± 0.000	0.053

Eth. T.A = Ethanolic extract of *Terminalia avicennioides*
Aq. T.A = Aqueous extract of *Terminalia avicennioides*
Eth. C.S = Ethanolic extract of *Cassia sieberiana*
Aq. C.S = Aqueous extract of *Cassia sieberiana*
Note: Data are expressed as mean ± SEM of duplicate experiments.

3.2 Acute toxicity test

The limit dose used (2000 mg/kg) did no produce mortality in the mice for the both the ethanolic and aqueous extracts. There were no gross behavioural changes such as loss of appetite, hair erection, tremors, convulsions, diarrhea and other signs of toxicity manifestation.

3.3 Acetic acid-induced writhing

Table 2: Effect of aqueous and ethanol extracts of *Cassia sieberiana* and *Terminalia avicennioides* on acetic acid-induced writhing in mice

S/N	Group	Number of writhing
1	Vehicle control (10 mL/kg)	33.000 ± 1.915
2	Aspirin (150 mg/kg)	5.167 ± 3.390*
3	ETH. T.A (200 mg/kg)	13.167 ± 4.423*
4	ETH. C.S (200 mg/kg)	12.667 ± 4.302*
5	AQ. T.A (200 mg/kg)	13.667 ± 5.835*
6	AQ. C.S (200 mg/kg)	19.167 ± 6.959*

Data are expressed as mean ± SEM and all data were analyzed using ANOVA followed by Bonferroni test for multiple comparisons for means. Number of animals per group (N) = 6. * Significantly different from control at $p \leq 0.05$.
In this study, there was significant $p \leq 0.05$ reduction in the number of writhing responses in each extract group when compared to the control

3.4 Formalin-induced paw edema

Table 3: Effect of aqueous and ethanol extracts of *Cassia sieberiana* and *Terminalia avicennioides* on formalin-induced paw edema in rats.

S/N	TIME	VEHICLE (10 mL/kg)	INDOMETHACIN (10 mg/kg)	ETH. T.A (200 mg/kg)	ETH. C.S (200 mg/kg)	AQ. T.A (200 mg/kg)	AQ. C.S (200 mg/kg)
1	Basal	0.618 ± 0.035	0.563 ± 0.046	0.588 ± 0.033	0.558 ± 0.052	0.614 ± 0.032	0.580 ± 0.037
2	30 mins	0.840 ± 0.054	0.912 ± 0.036	0.780 ± 0.028	1.058 ± 0.078	0.814 ± 0.043	0.913 ± 0.053
3	1 h	0.914 ± 0.029	1.293 ± 0.065	1.265 ± 0.061	1.188 ± 0.062	1.084 ± 0.068	1.038 ± 0.017
4	2 h	1.050 ± 0.056	1.160 ± 0.062	1.285 ± 0.073	1.285 ± 0.054	1.030 ± 0.062	1.113 ± 0.032
5	24 h	1.590 ± 0.070	1.445 ± 0.110	1.292 ± 0.080**	1.592 ± 0.086	1.638 ± 0.130	1.585 ± 0.070

Data are expressed as mean ± SEM and all data were analyzed using ANOVA followed by Bonferroni test for multiple comparisons for means. Number of animals per group (N) = 6. ** Significantly different from control at $p \leq 0.01$.
In the present study, there was significant $p \leq 0.01$ decrease in inflammation with the ETH. T.A at 24 h when compared with the vehicle control.

3.5 Chronic inflammation
3.5.1 Changes in Body Weights

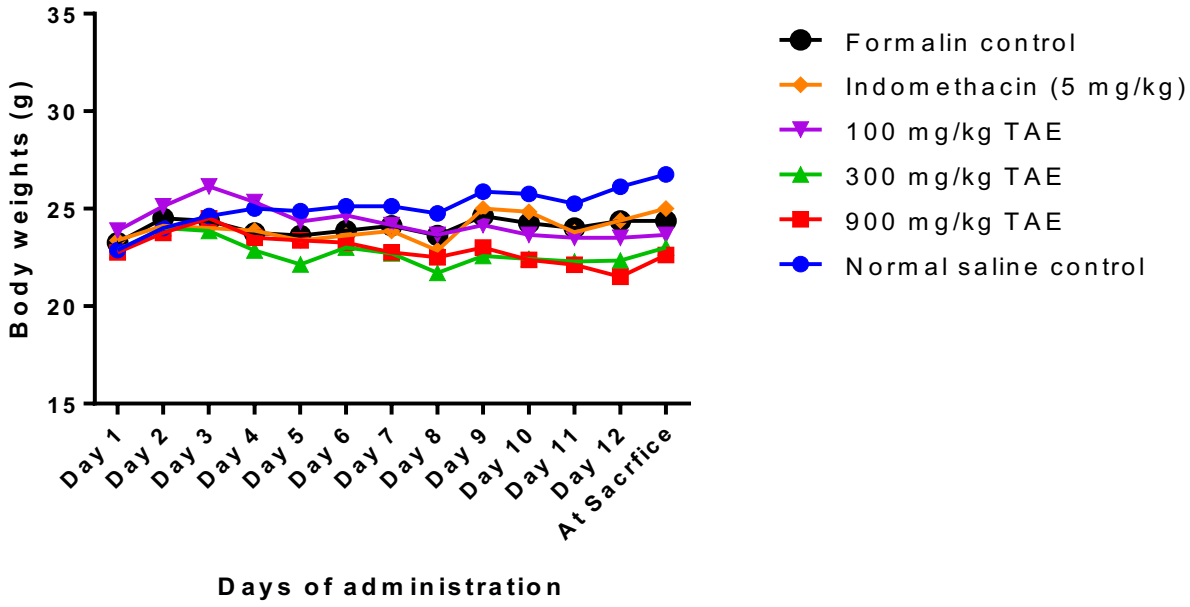

Fig 1: Changes in body weight of the mice
There was no significant change in body weight across the treated groups when compared with either the normal saline control or the formalin-injected control over the 10-day study period.

3.5.2 Changes in paw volume

Table 4: Changes in paw volumes of the mice before and after treatment

Group	Paw volume (before treatment)	Paw volume (after treatment)	Differences (% changes)
Normal saline	0.145 ± 0.012	0.229 ± 0.008 [b]	8.375
Formalin control	0.147 ± 0.009	0.410 ± 0.011 [a]	26.250
Indomethacin (5 mg/kg)	0.146 ± 0.009	0.273 ± 0.026 [ab]	12.708
900 mg/kg TAE	0.146 ± 0.010	0.308 ± 0.027 [ab]	16.125
300 mg/kg TAE	0.145 ± 0.010	0.306 ± 0.018 [ab]	16.071
100 mg/kg TAE	0.146 ± 0.010	0.375 ± 0.008 [b]	22.875

Values are expressed as Mean ± SEM of eight mice, mL. Differences = (paw volume at sacrifice-paw volume before treatment) * 100

[a]$p < 0.01$ as compared with normal saline control

[b]$p < 0.01$ as compared with formalin control

A significant $p < 0.01$ increase in paw volume was observed for both the formalin-injected and the treated groups from the first to the tenth day of treatment when compared to the normal saline control.

3.5.3 Changes in paw weight

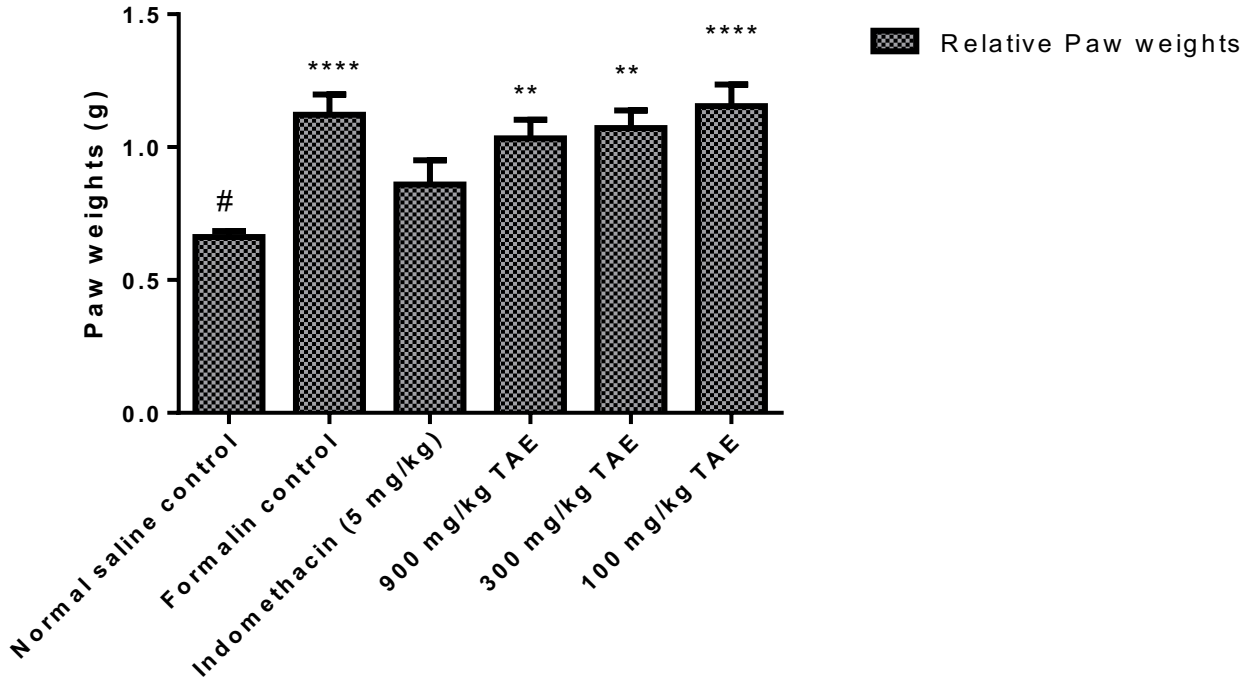

Fig 2: Relative paw weight at sacrifice

Values are expressed as Mean ± SEM.

****$p < 0.0001$ as compared with normal saline control

**$p < 0.01$ as compared with normal saline control

#$p < 0.0001$ as compared with formalin control

There was significant increase in relative paw weight at the time of sacrifice across the treated groups and the formalin-injected group when compared to the normal saline control. There was no significant change in relative paw weight across the treated groups when compared to the formalin control.

3.5.4 Measurement of nitrite

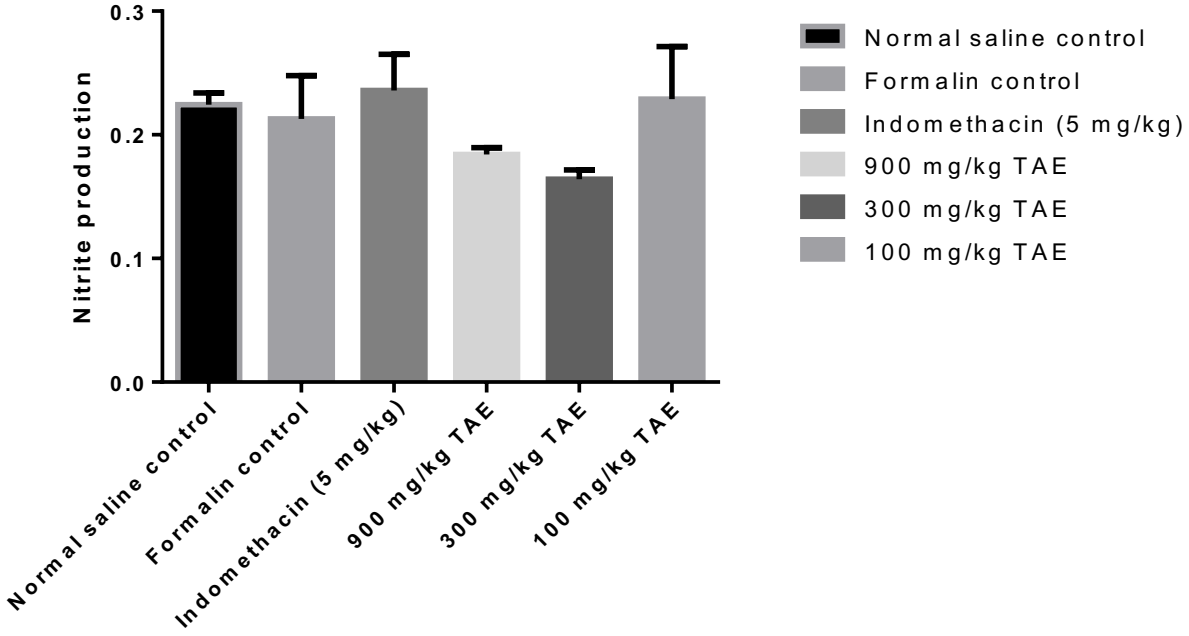

Fig 3: Nitrite production from tissue homogenate

Values are expressed as Mean ± SEM. Values are non-significant at $p > 0.05$ when compared to either the normal saline control or the formalin control

4.0 Discussion

In the present study, the analgesic and anti-inflammatory effects of the ethanol and aqueous extracts of two plants *Cassia sieberiana and Terminalia avicennioides* were evaluated. Focus was on the ethanol extract of *T. avicennioides* which showed the highest content of phenols in terms of gallic acid equivalents (GAE) and also showed a significant decrease in formalin-induced paw edema in rats after 24 h of treatment.

The ethanol extracts of the plants and the aqueous extract of *Terminalia avicennioides* showed good content of phenols and may likely possess good antioxidant activity. Higher phenolic contents were displayed by the ethanol extract signifying that the phenolics are less polar thereby better extracted with ethanol.

In the acetic acid-induced writhing, there was a significant ($p \leq 0.05$) decrease in writhing response across all the treated groups and the Aspirin treated group. The decrease was more evident in the group treated with *C. sieberiana* with the aqueous extract showing the highest activity thus, suggesting that *C. sieberiana* had more analgesic property.

In the formalin-induced paw edema in rats, there was increase in paw volume across the groups after 30 min of induction of inflammation which continued to increase through the study duration. The group treated with the ethanol extract of *T. avicennioides* showed a decrease in inflammation as compared to other treated groups and significantly different ($p < 0.01$) from the vehicle control after 24 hours thus, the presence of anti-inflammatory activity.

There was no significant change in body weight as compared with the two controls (normal saline and formalin) over the period of study showing that the ethanol extract of *T. avicennioides* at the doses used had little or no effect on body weight although slight decrease in weight were observed from the fourth day of treatment in both the extract and indomethacin treated groups. Increase in paw volume was observed after formalin injection on the first and third days which lasted through the period of study. The indomethacin group showed significant ($p < 0.01$) reduction in inflammation throughout the study period. Indomethacin being a potent anti-inflammatory agent which works via inhibition of cyclooxygenase, an enzyme that catalyzes the production of prostaglandins through the arachidonic pathway. Prostaglandins are released during an inflammation and they mediate the inflammatory pathway. There was also reduction in inflammation across the treated groups which was significant ($p < 0.01$) as compared with the formalin control.

Changes in relative paw weight was demonstrated as significant ($p < 0.01$) increase in relative paw weights were observed across the treated and the formalin-injected groups as compared to the normal saline group. Normal saline being an isotonic fluid is absorbed easily into the tissue thereby not affecting the paw weight.

The nitric oxide production calculated in terms of nitrite using a standard plot of sodium nitrite demonstrated a reduction on nitrite production in the 300 and 900 mg/kg extract treated groups. The 900 mg/kg group gave the least value for nitrite. There was a slight increase in nitrite production in the 100 mg/kg group which is an indication of low anti-inflammatory activity.

From the present study, it can be concluded that *Terminalia avicennioides* stem bark extract possesses anti-inflammatory activity but there is need for more intense studies to prove such. If proven, it can serve as a source of new lead compounds for the management of inflammatory conditions

REFERENCES

1. OECD (Organization for Economic Cooperation and Development). OECD Guidelines for Testing of Chemicals 423: Acute Oral Toxicity-Acute Toxic Class Method, 1st Adoption; Organization for Economic Cooperation and Development, Paris, France, 17 December 2001.
2. Rokia Sanogo. Afr. J. Tradit. Complement. Altern. Med. 2011; 8(S):90-96.
3. Narcotic drugs estimated world requirements for 2008, statistics for 2006. New York: United Nations pubns. 2008. P.77.
4. Salihu T, Olukunle JO, Adenubi OT, Mbaoji C and Zarma MH South African Journal of Botany 2018;118: 33-43
5. Arsenault, Patrick; Wobbe, Kristin; Weathers, Pamela. Current Medicinal Chemistry. 2008; 15 (270: 2886-2896
6. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed. Proc. 1959; 18: 418-420.
7. Willcox, Merlin. traditional medicinal plants and malaria. Crc press 2004; p. 231
8. Mensor LL, Fabio SM, Gilda GL et al. Phytotherapy Research. 2001; 15(2)