

## Original Research Article

# Analgesic, anti-inflammatory and toxicological effects of Vigornatural herbal powder in experimental animals

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### Abstract

**Purpose:** To determine the analgesic, anti-inflammatory and toxicological effects of Vigornatural herbal powder, derived from the leaves of *Nicotiana tabacum* L. (Solanaceae) and the seeds of *Buchholzia coriacea* Eng. (Capparaceae).

**Methods:** Analgesic and anti-inflammatory effects of the herbal powder were assessed using acetic acid-induced writhing and egg-albumin-induced edema models, respectively. Drugs were administered orally to animal groups ( $n = 4$ ) at a dose of 400 mg/kg using paracetamol and Tween-80 as positive and negative controls, respectively in writhing test, while in egg albumin model, drugs were administered at a dose range of 100 - 400 mg/kg using diclofenac sodium and Tween 80 as controls respectively. Acute toxicity was evaluated using modified Lorke's protocol. Sub-acute toxicity was investigated in Wistar rats after 14 days of oral administration of 400 mg/kg per day via its effect on hematological and serum biochemical parameters. Qualitative phytochemical analysis was carried out using standard protocols.

**Results:** The herbal powder gave a median lethal dose ( $LD_{50}$ ) > 5000 mg/kg and significantly provoked 38 % inhibition of acetic acid-induced writhing in mice at 400 mg/kg dose, relative to negative control ( $p < 0.05$ ). It also significantly inhibited both phases of egg-albumin-induced edema with 9.5 and 16.5 % inhibition at 1st and 4th hours, respectively when compared to standard diclofenac which gave 4.2 and 17.3 %, respectively ( $p < 0.05$ ). There was no significant difference between the treated and control groups with regard to all hematological and biochemical parameters tested after 14 days. Qualitative phytochemical tests showed the presence of alkaloids.

**Conclusion:** Vigornatural herbal powder possesses fairly good analgesic and anti-inflammatory properties and does not have any significant adverse effect on hematological, hepatic and renal functions. Further studies to develop it as a potential novel analgesic and anti-inflammatory pharmacotherapeutic agent is recommended.

**Keywords:** Analgesic, Anti-inflammatory, Toxicology, Hematology, Serum biochemistry, Herbal medicine

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## INTRODUCTION

Pain and inflammatory disorders are responsible for most hospital visits worldwide, with chronic musculoskeletal pain affecting 20 - 33 % of the world population (about 1.7 billion people) according to World Health Organization (WHO) [1]. Pain induced by diseases of the nerves (neuropathic pain) accounts for 6.9 – 10 % of the burden [2]. About 20 % of the adult population have pain in their internal organs due to pressure, injury, surgery, ulcers, and other conditions as well as economic and social factors, with abdominal symptoms being most prevalent in children and adolescents [3]. Despite the tremendous advances in medical and pharmaceutical sciences in drug discovery, pain and inflammatory disorders remain a huge burden to humanity. In West Africa and Nigeria in particular, the unbearable burden has led to the abuse of Prescription Only Medicines (POMs) like opioids, with the resultant mental debilitation posing even more danger to society [4].

Non-steroidal anti-inflammatory drugs (NSAIDs) have been indispensable in treatment of osteoarthritis, rheumatoid arthritis, oncologic pain, acute and chronic pain and inflammation. Non-selective NSAIDs work by inhibiting both type 1 and 2 cyclo-oxygenases (COX) enzymes to bring about their analgesic and anti-inflammatory efficacies, leaving behind gastrointestinal ulceration and kidney failure as major adverse effects following prolonged use. The development of Celebrex (Celecoxib), a selective type 2 COX enzyme inhibitor has helped to overcome these adverse effects to some extent. Celebrex however, with other COX-2 inhibitors has serious adverse cardiovascular effect such as thrombosis on prolonged use [4].

Unlike toxicity, end-organ damage, high cost, abuse/addiction, hospital/pharmacy visit stress, and many more associated with NSAIDs, opioids and other orthodox medicines, herbal remedies are readily available, have fewer adverse and side effects due to their 100 % organic origin and are highly efficacious due to their biocompatibility [5]. There's, therefore, the need to push search for better analgesic and anti-inflammatory agents towards herbs in a bid to find safer remedies with sufficient bioactivity to alleviate the burden on humanity.

Toxicology and safety constitute essential roles in the discovery and development of herbal medicines. A number of protocol documents on safety and toxicity testing of herbal medicinal products have been put forward. Depending on the protocol used, hematological parameters: full

blood count (FBC), hemoglobin level (Hb) packed cell volume (PCV), and biochemical parameters (serum biochemistry) for organ function assessment are pivotal. Despite the wide use of herbal medicines, many of the claims on pharmacological properties have not been validated scientifically. Toxicity and safety data have also not been provided.

*Vigornatural* herbal powder, a product of Vip Connect Nig. Ltd., based in Nigeria, is a potent analgesic, anti-inflammatory and anti-ulcer herbal product used widely in the Southeastern part of Nigeria for relief of pain, inflammatory disorders and peptic ulcer. It is composed mainly of *Nicotiana tabacum* L. (Solanaceae) and seeds of *Buchholzia coriacea* Eng. (Capparaceae). This study therefore aims to determine the analgesic activity claims and the safety profile of *Vigornatural* Herbal Powder (VNHP), an herbal drug used extensively in Southeastern Nigeria for the treatment of peptic ulcer, pain and inflammatory disease.

## EXPERIMENTAL

### Animals

Adult Wistar rats of both sexes weighing 200 – 300 g, and adult Swiss albino mice of both sexes, weighing 22 – 35 g, were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka. The animals were housed in clean steel cages with good ventilation and maintained at ambient temperatures. They were fed with mixed pelleted feed (Top Feeds) and given access to clean portable water at all times. All the research protocols used in this work were approved by the University of Nigeria Ethics Committee on the use of experimental animals (approval no. NHREC/05/01/2008B) and conducted in line with internationally accepted principles for laboratory animals' use and care in the European Economic Community guidelines [6].

### Equipment and instruments

Microcapillary tube (Marienfeld, Germany), microhematocrit centrifuge (Hawksley, England), microhematocrit reader and hemocytometer set (Hawksley, England), automatic pipettes (Superfit Equip, Ames), light microscope (Leica Inc. USA), slides and coverslips (Surgifriend Medicals, England), Quimica Clinica Aplicada AST Test Kit (QCA, Spain). All reagents for phytochemical analysis were prepared from pure samples of various inorganic chemicals (Sigma Aldrich).

### Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out on VNHP using customary procedures [7]. Sample of VNHP was donated by the manufacturer, VIP Connect Ltd.

### Acute toxicity assay

Acute toxicity test on VNHP was done using a modified form of Lorke's method [8]. Adult (22 - 35 g) were used for this assay. Test sample was administered orally to the groups in two stages. In the first stage, three groups of animals (n = 3) each were administered with a 3 % v/v Tween-80 solution of the powdered product at 10, 100 and 1000 mg/kg doses respectively and observed for 24 h.

In the 2nd segment, further 1600, 2900 and 5000 mg/kg doses respectively of the product were administered to another three groups (n = 2). The animals were again monitored for 48 h.

### Hematological tests

The test sample was evaluated for its effect on hematological parameters in adult Wistar rats after 14 days of oral administration of 400 mg/kg to the animals (n = 3), using selected standard protocols [9]. Blood samples were withdrawn from each animal through a temporary cannula without anesthesia. The 400 mg/kg dose was calculated based on usual recommended dose. Control group received 1 mL 3 % v/v Tween-80 each.

### Measurement of packed cell volume

Packed cell volume (PCV) was measured using Microhematocrit method [9]. Microcapillary tubes, sealed at one end, were nearly filled with blood samples and whirled in a micro-hematocrit centrifuge set at 10,000 revolutions per min for 5 min. It was then measured using a microhematocrit reader.

### Determination of hemoglobin concentration (Hb)

The hemoglobin concentrations were determined by Cyanomethemoglobin method [10]. Each of the blood samples and standard (0.02 mL each) was mixed with 5 mL of Drabkin's hemoglobin reagent. The mixture was incubated for 20 min and hemoglobin concentration read at 540 nm against a reagent blank on a Semi-automated Clinical Chemistry Analyzer.

### Determination of erythrocyte count

Red blood cell count was determined using the Hemocytometer method as previously described [9]. A 1:200 dilution of the blood sample was loaded onto a Neubauer counting chamber and all red blood cells were counted using a light microscope at x40 objective.

The number of cells counted for each sample was multiplied by 10,000 to obtain the erythrocyte count per microliter of blood.

### Evaluation of total leukocyte count

The total leukocyte count was determined using hemocytometer method as previously described [9]. A 1:20 dilution of blood sample was prepared and added to Neubauer counting chamber and cells were counted using a light microscope at x10 objective.

### Determination of differential leukocyte count (TLC)

Leishman protocol as previously described [9] was followed in determination of differential leukocyte count. Thin, air-dried smear of each of the blood samples was stained with Leishman stain and examined with a light microscope. The value was multiplied by 50 to get TLC per microliter of blood.

### Serum biochemistry

Based on the usual dose recommended by the producer, animal groups (n = 3) were treated orally with a 400 mg/kg dose daily for 14 days while keeping other conditions relatively constant (regular feeding, free access to water, constant relative humidity and temperature).

Control group received 1 mL 3 % v/v Tween-80. After 14 days, blood was withdrawn from each animal through a temporary cannula without anesthesia and whirled briskly in a centrifuge to obtain serum used for the assay.

### Evaluation of serum aspartate amino transferase (AST) activity

The Reitman-Frankel colorimetric method as described previously [10] was followed using the Quimica Clinica Aplicada (QCA) AST kit. The serum AST activity level of the sample against a deionized water blank was read using the Diatek Biochemistry Analyzer set at the AST-QCA Program Mode and the results were recorded.

### Assessment of serum alanine amino transferase (ALT) activity level

Reitman-Frankel colorimetric method described previously [10] was followed. Incubation was done at 37 °C for 5 min. The colour developer (Reagent B) and NaOH solution (Reagent C) were added appropriately. Serum ALT activity concentration (IU/L) of sample against deionized water blank was read using Diatek Biochemistry Analyzer set at ALT-QCA Program Mode and the result was recorded.

### Determination of serum alkaline phosphatase (ALP) activity level

The TECO ALP assay kit was used for this assay and thymolphthalein monophosphate method described previously [11] followed. The reagent mixtures were incubated at 37 °C for 10 min. After the incubation, 1.0 mL of ALP colour developer (Reagent B) was added appropriately after which ALP activity concentration (IU/L) on ALP-TECO Program Mode of Diatek Blood Biochemistry Analyzer was read and results was retrieved.

### Determination of serum total proteins

Assay of serum proteins was achieved using Randox total protein and albumin test kits. The Direct Biuret method [12] was used as the protocol. Biuret Reagent (10 mL each) was mixed with 0.02 mL each of the serum sample and standard reagent and left to stand for 30 min at 25 °C. Total protein levels were read using Diatek Biochemistry Analyzer, set at Randox Total Protein Program Mode and the result was recorded.

### Serum albumin assay

Bromocresol green method [12] was used to assay serum albumins. Bromocresol green reagent was added to 0.01 mL of each of the serum samples and standard and allowed to stand for 5 min at 25 °C. Albumin levels of the samples against reagent blank were read using the analyzer, set at Randox Albumin Program Model and the results were retrieved. The difference between total protein and total albumin was considered the globulin level [12].

### Assay of serum total bilirubin

Serum total bilirubin determination was done with Randox Bilirubin test kit using modified Jendrassik and Grof method [9]. The total bilirubin levels of samples against blank were read using the analyzer, set at RANDOX Total Bilirubin Program Mode (wavelength – 578 nm;

multiplication factor – 10.8) and the results were recorded.

### Assay of serum creatinine

The modified Jaffe method [13] was carried out using Quimica Clinica Aplicada (QCA) test kit. Equal volumes (3 mL each) of reagent's A and B (alkaline solution and picric acid solution) were mixed to make 6 mL of reagent W. For each serum sample, 0.1 mL (100 µL) of serum sample was properly mixed with 1.0 mL of reagent W. A stopwatch was started and absorbance at the 20<sup>th</sup> sec and 80<sup>th</sup> sec ( $A_{20s}$  and  $A_{80s}$ ) against a distilled water blank at 510 nm was read and recorded. A 0.1 mL (100 µL) of Reagent C (standard) was also mixed with 1.0 mL of Reagent W and again shaken gently to mix properly. The absorbance at 20<sup>th</sup> and 80<sup>th</sup> sec against distilled water (blank) at 510 nm was also read. The mean of the standards was computed and calculation of serum creatinine level (C) was done using Eq 1.

$$C \text{ (mg/dL)} = \frac{\Delta Sa(A_{80s} - A_{20s})}{\Delta St(A_{80s} - A_{20s})} \times 2 \dots\dots\dots (1)$$

where Sa is sample and St is standard.

### Assay of plasma/serum urea

The modified Berthelot reaction method [13] was used for this analysis, using the Dialab Urea test kit. The reagents used include Mixture A (containing Sodium nitroprusside, Sodium salicylate, Ethylenediamine tetraacetic acid (EDTA) and Phosphate buffer), Solution B (containing Phosphate buffer, NaOH and Sodium hypochlorite), Solution C (containing Urease), and the Standard (composed of aqueous solution of urea equivalent to 50 mg/dL). After mixing the reagents and incubating appropriately at room temperature for ten min. Urea concentration was read using Diatek Biochemistry Analyzer set in UREA-DIALAB Program Mode.

### Analgesic activity test

The acetic acid-induced writhing model [14] was used to evaluate the inhibition of peripheral nociception as a function of analgesic activity of the test sample. Adult Swiss albino mice of both sexes (22 - 25 g) were randomly selected and divided into three groups (n = 4). The VNHP was dispersed in 3 % v/v Tween 80 and given to the animals orally in the first group at 400 mg/kg. The 400 mg/kg dose was calculated based on the usually recommended dose. To the animals in group 2, 40 mg/kg of paracetamol was orally administered while group 3 animals received 0.2

mL of 3 % v/v Tween 80 orally. One hour after oral administration, a 0.7 % aqueous solution of acetic acid was injected into the peritoneal region of the animals at a dose of 10 ml/kg. Numbers of writhes within 5 min were counted for each animal starting from 5 min after the intraperitoneal injection.

**Anti-inflammatory activity assay**

The anti-inflammatory activity assay protocol used was the egg albumin edema method. In the test sample, a suspension of VNHP in 3 % v/v Tween 80 was made and administered in doses of 100, 200 and 400 mg/kg orally to the first 3 groups (n = 4). In control groups, Wistar rats of both sexes and equivalent weights were divided into two groups of four animals each (the 4 and 5<sup>th</sup> groups). The 4th group received 0.2 mL of the vehicle (3 % v/v Tween 80) while the 5th group received 5 mg/kg diclofenac sodium orally. One hour later, acute inflammation was provoked by injecting 0.1 mL of fresh egg albumin into the left hind paw of each animal. The percentage inhibition (I) of edema, as a function of anti-inflammatory activity was computed according to Eq 2 [14].

$$I \text{ (\%)} = \frac{\{V_0 - V_{t(\text{control})}\} - \{V_0 - V_{t(\text{test})}\}}{\{V_0 - V_{t(\text{control})}\}} \times 100 \dots\dots\dots (2)$$

where  $V_t$  is the edema volume of the test sample group at a time,  $t$ , and  $V_0$  is the edema volume of negative control group at the same time. Oedema volume was measured with a plethysmometer at 0 hour and then every hour for 4 hours after administration of egg albumin.

**Statistical analysis**

All the data were analyzed using One-way analysis of variance (ANOVA) followed by

Dunnett’s multiple comparisons post-hoc test. The software used was GraphPad Prism version 7.0. The values are expressed as mean ± standard deviation.  $P < 0.05$  was adjudged statistically significant.

**RESULTS**

**Qualitative phytochemical profile**

The secondary metabolites present in VNHP powder are shown in Table 1.

**Acute toxicity**

The animals did not display any sign of drug toxicity or illness after treatment with the VNHP within 48-hour observation period, after the initial oral administration of a maximum of 1,000 mg/kg. Neither was any sign of toxicity seen even after increasing the dose to 5,000 mg/kg nor was death recorded.

**Hematological data**

The effects of the VNHP on the hematological parameters of animals after 14 days of treatment with 400 mg/kg of the VNHP are shown in Table 2. There is no significant difference between the treated and control groups.

**Table 1:** Qualitative phytochemical composition

Phytochemical class	Presence
Alkaloid	+++
Flavonoid	-
Saponin	-
Tannin	-
Steroid	-

**Note:** - Not detected; +++ highly abundant

**Table 2:** Effect of the VNHP on hematological parameters (mean ± SD)

Parameter	Treated	Control
PCV (%)	35.6±5.1	42.6±3.2
Haemoglobin (g/dL)	13.20±1.8	14.7±0.1
RBC count (10 <sup>6</sup> /μL)	4.9±1.1	8.4±1.3
Total WBC count (10 <sup>3</sup> /μL)	14.7±0.2	14.1±2.6
Abs. lymphocyte count (10 <sup>3</sup> /μL)	6.4±2.5	8.6±2.8
Abs. neutrophil count (10 <sup>3</sup> /μL)	7.7±1.8	4.7±1.9
Abs. Eosinophil Count (10 <sup>3</sup> /μL)	0.4±0.3	0.5±0.2
Abs. Monocyte count (10 <sup>3</sup> /μL)	0.3±0.2	0.3±0.1
Percentage lymphocytes (%)	43.5±16.3	60.3±15.2
Percentage Neutrophils (%)	52.0 ± 12.7	34.0 ± 15.7
Percentage Eosinophil (%)	2.5±2.1	3.3±0.6
Percentage Monocytes (%)	2.0 ± 1.4	2.3±0.6

PCV= Packed cell volume; RBC = Red blood cell; WBC = White blood cell; Abs.= absolute

### Serum biochemical data

After administering a 400 mg/kg daily dose of VNHP to the animals for 14 days, the serum biochemical data obtained compared appreciably with the control as shown in Table 3. There is no significant difference between the treated and control groups.

**Table 3:** Effect of the *Vigornatural* herbal Powder on serum biochemical data (Mean  $\pm$  SD)

Parameter	Treated	Control
Total Proteins (g/L)	69.3 $\pm$ 8.7	73.5 $\pm$ 11.7
Albumins (g/L)	38.9 $\pm$ 1.4	39.0 $\pm$ 1.7
Globulins (g/L)	30.4 $\pm$ 8.0	34.6 $\pm$ 10.3
AST (IU/L)	32.8 $\pm$ 2.1	47.4 $\pm$ 12.2
ALT (IU/L)	14.2 $\pm$ 4.1	19.0 $\pm$ 2.3
ALP (IU/L)	39.3 $\pm$ 5.8	46.9 $\pm$ 3.0
Total Bilirubin (mg/dL)	0.6 $\pm$ 0.4	0.5 $\pm$ 0.3
Urea (mg/dL)	44.6 $\pm$ 2.0	43.3 $\pm$ 0.3
Creatinine ((mg/dL)	1.0 $\pm$ 0.4	0.8 $\pm$ 0.1

AST = aspartate amino transferase; ALT= alanine amino transferase; ALP = alkaline phosphatase

### Peripheral analgesic activity

The percentage inhibition of acetic acid-induced writhing, as a function of peripheral analgesic activity, is shown in Table 4.

**Table 4:** Inhibition of VNHP in acetic acid-induced writhing in mice (mean  $\pm$  SD)

Group	No. of writhes	Inhibition (%)
VNHP ((400 mg/kg)	23.67 $\pm$ 3.84*	40.33
Paracetamol (40 mg/kg)	18.00 $\pm$ 2.31**	54.63
Negative control	39.67 $\pm$ 2.60	

\* $P < 0.05$ ; \*\* $p < 0.01$  relative to negative control

### Dose-dependent inhibition of egg albumin-induced edema

The percentage inhibition of edema induced by egg albumin, as a measure of anti-inflammatory activity, is shown in Table 5. The herbal powder evoked 11 % inhibition of edema at 100 mg/kg after 3 h and 14.7 % at 200 mg/kg after 4 h. There was no significant activity at 200 mg/kg after 1 h of edema induction while the highest activity (16.51 %) occurred at 400 mg/kg after 4 h of edema induction with fresh egg albumin. The same percent inhibition was recorded with 5 mg/kg diclofenac sodium after 4 h of edema induction.

## DISCUSSION

The qualitative phytochemical screening showed the presence of primary alkaloids in the VNHP,

suggesting that alkaloids are responsible for the pharmacological properties of this product. Alkaloids are well known to possess potent and diverse pharmacological effects such as analgesia (e.g., opioids), anti-cancer (e.g., aborigine, skimianine) and antimalarial (e.g., artemisinin) [16].

**Table 5:** Percentage inhibition of VNHP in egg albumin-induced edema (mean  $\pm$  SD)

Treatment	Dose (mg/kg)	Inhibition of edema (%)			
		1 h	2 h	3 h	4 h
NVHP	100	3.2	5.4*	11.0***	7.6**
	200	0.0	6.3*	9.3*	14.7***
	400	9.6***	11.8**	13.8***	16.5***
Diclofenac	5	4.4	12.3**	16.6***	17.3***

\* $P < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

From the result of the acute toxicity assay, the animals exhibited no clinical sign of toxicity, within the 48-hour observation period. No sign of toxicity was seen even after increasing the dose to 5000 mg/kg. The LD<sub>50</sub> is, therefore, much greater than 5000 mg/kg. According to the Loomis and Hayes classification, an LD<sub>50</sub> > 5000 mg/kg is rated practically nontoxic [17]. This indicates that the investigated herbal product has a good safety profile. After administering 400 mg/kg daily dose of the VNHP to the animals, there was no marked difference in the values of the hematological parameters studied between the treated and control groups. This indicates that the drug had no substantial adverse effects on the hematological parameters.

There was, however, an insignificant decrease in the PCV, hemoglobin concentration and total RBC count in the treated group, relative to the control. This could be attributed to minor destruction of the erythrocytes. Though the decrease was inconsequential, concurrent administration of blood supplements may be recommended. The liver function was appraised in the animals, after administering 400 mg/kg of VNHP daily for 14 days, by determining the activities of the serum AST, ALT, ALP and bilirubin which are enzymes naturally present in large quantities in the cytoplasm [18]. In case of an abnormal or diseased state of the liver, these enzymes escape into the bloodstream in quantities proportional to the degree of the liver injury [18]. A high serum concentration of AST indicates bruising, trauma, necrosis, infection, or neoplasia of the liver or muscle [18]. The serum biochemical data obtained from the liver function tests suggest no impairment in the hepatic and renal functions of the animals. For the kidney function test, the serum creatinine and urea

levels are paramount in ascertaining renal function in clinical settings. High levels of these substances in the blood indicate impaired functioning of the kidneys. The blood urea and creatinine concentrations of animals, after 14 days of oral treatment with 400 mg/kg dose, as a function of the kidney condition, showed no significant difference between the treated and the control animals, suggesting that *VNHP* was not toxic to the liver. The *VNHP*, at 400 mg/kg, provoked 40.33 % inhibition of writhing induced by acetic acid, suggesting fairly good peripheral analgesic activity. This result compares fairly well with that of the standard analgesic, paracetamol at 40 mg/kg which had an inhibition of 54.63 %. Writhing is an explicit reaction to the severe ache triggered by irritant substances through nociceptors, typified by incidents of withdrawal of the abdomen and extension of the rear limbs. The stimuli passed on to the central nervous system in rejoinder to the pain resulting from the irritation, trigger the discharge of mediators such as prostaglandins which aggravate sensitivity to the nociceptors [19]. This result, therefore, suggests inhibition of prostaglandin synthesis as the possible mode of action of the test drug. For the egg albumin model, the result suggests a dose-dependent mild anti-inflammatory effect, similar to that of the standard, diclofenac sodium at 5 mg/kg. The egg albumin-induced edema is known to be made up of acute and chronic phases. The first phase is mediated by the release of histamine and serotonin which begins in the 1<sup>st</sup> hour and reaches the peak in the 2<sup>nd</sup> h after the subcutaneous injection [15]. Migration of leukocyte cells also plays an important role. The later phase (3<sup>rd</sup> - 4<sup>th</sup> h) is mediated by bradykinin, leukotrienes, leukocyte infiltrations, and biosynthesis of prostaglandin by inducible cyclooxygenase [15]. It is evident from Table 5 that the test drug at 400 mg/kg dose fairly inhibited both phases of inflammation and inhibited histamine better than diclofenac in the first hour. Diclofenac is a known prostaglandin inhibitor.

## CONCLUSION

*Vigornatural Herbal Powder* is a relatively safe drug in animal models with no significant acute and sub-acute toxic effects on the liver and kidneys as well as on hematological parameters. It also possesses significant analgesic and anti-inflammatory properties. However, it should be further investigated *in vivo* for its analgesic and anti-inflammatory properties.

## DECLARATIONS

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### Funding

None provided.

### Ethical approval

None provided.

### Availability of data and materials

The data that support the results of this study are available from the Departments of Pharmaceutical & Medicinal Chemistry, and Pharmacology & Toxicology, University of Nigeria Nsukka, and can be obtained from the authors upon request and with the permission of Vip Connect Nig. Ltd.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Fabian I Eze and Fabian U Ozioko conceived and designed the experiment; Fabian U Ozioko, Jacob U Inuaeyen, Stephanie O Emmanuel, Ezinne B Ugwu, Onyinyechukwu Y Ugonabo and Chisom M Amoke carried out the laboratory experiments; Jacob U Inuaeyen and Stephanie O Emmanuel drafted the original manuscript; Peter E Ikechukwu, Fabian I Eze and Chisom M Amoke analyzed and interpreted the data, Chisom M Amoke assisted in responding to reviewer's comment, while Fabian I Eze proofread and reviewed the manuscript and supervised the work. All authors read and approved the final manuscript.

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