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Alterations in haematological indices of indomethacin-ulcer*ated rats* treated with *Persea americana* seed and *Bryophyllum pinnatum* leaf ethyl acetate fraction

Emeka Sabastine Asiwe [1,*], Prince Chimaobi Ejiogu [2], Uchechi Emmanuela Enenebeaku [1], Peter Onyedikachi Opara [1] and Obinna Michael Oguoma [2]

[1] *Department of Biological Sciences, University of Agriculture and Environmental Sciences, Umuagwo, Imo State, Nigeria.*
[2] *Department of Biochemistry, Federal University of Technology Owerri, Imo State, Nigeria.*

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Abstract

This study determined the alterations in haematological parameters of indomethacin-ulcerated rats treated with *P. americana* seed (PAS) and *B. pinnatum* leaf (BPL) ethyl acetate fraction. Fifty (50) healthy Wistar albino rats were randomly assigned into ten (10) groups of five (5) animals each according to body weight (100-120 g). Animals in group I served as normal control; the rats in groups III-X were pre-treated with 20 $mgKg^{-1}$ b. wt. Omeprazole (STD), 400 $mgKg^{-1}$ b. wt. PAS, 400 $mgKg^{-1}$ b. wt. BPL, 400 $mgKg^{-1}$ b. wt. PAS + BPL (1:1), 400 $mgKg^{-1}$ b. wt. PAS + BPL (1:2), 400 $mgKg^{-1}$ b. wt. PAS + BPL (1:3) respectively for 21 days. Thereafter, animals in groups II-X were induced for gastric ulcer by intubation of 30 $mgKg^{-1}$ b. wt. indomethacin after being fasted for 24 hours. The animals were sacrificed after 4 hours and the complete blood count was determined using an automated hematology analyzer. Results obtained from the study showed significant ($p<0.05$) elevation in the total and differential white blood cell count of Wistar rats in the indomethacin-induced gastric ulcer control group and ethyl acetate fraction treated groups. Indomethacin, PAS, and BPL ethyl acetate fraction and the combinations did not cause marked changes in red blood cell indices. However, platelet count was significantly ($p<0.05$) reduced by the combinations of the *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction. These findings demonstrated that indomethacin induction resulted in a significant increase in the total white blood cell and platelet count when matched with the control group; this was similarly seen in PAS+BPL (1:3) and PAS+BPL (3:1) combinations of PAS and BPL. The result is suggestive of a compromise in the hemostatic capability of the blood in rats treated with some combinations of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction.

Keywords: *Persea americana*; *Bryophyllum pinnatum*; Indomethacin; Gastric ulcer; Haematology

1. Introduction

Gastric ulcers are one of the most common types of peptic ulcers and mainly occur in the stomach and proximal duodenum. It is marked by a sore lining in the stomach or duodenum due to a breakdown in the stomach's defense against gastric acidity [1]. Peptic ulcers develop due to an imbalance in causative factors and defensive factors favoring aggressive factors. Some gastric aggressive factors include lifestyle patterns such as diet, smoking, alcohol consumption, and non-steroidal anti-inflammatory drugs (NSAIDS) use; biotic and biochemical changes such as Helicobacter pylori infection, reactive oxygen species (ROS), hydrochloric acid (HCl), pepsin, refluxed bile, leukotrienes (LTs) [2]. The body counteracts this effect through a synergy of homeostatic mechanisms which include the mucosal blood flow, cell renewal and migration, mucus-bicarbonate barrier, non-enzymatic and enzymatic antioxidants, prostaglandins (PGs), and some growth factors. Previous population-based studies have pitched the estimated lifetime global prevalence of gastric ulcer disease at 5–10%, prevalence of 12 - 25% for the sub-Saharan Africa region, and of 0.1–0.3% annual incidence in Western countries [3, 4, 5]. The incidence and risk of gastrointestinal dysfunctions increase with age [6,7]. Also, higher prevalence rates have been reported for countries with high rates of *H. pylori* infection. H. pylori infections

* Corresponding author: Asiwe, Emeka S

have been associated with haematological dysfunction and alteration of hematinic factors such as micronutrient deficiency (iron, and vitamin B12) [8, 9].

NSAIDS use has been strongly implicated in the promotion of gastric ulceration through inhibition of cyclo-oxygenase (COX-1) enzyme expression; cyclo-oxygenase (COX-1) and (COX-2) catalyze the synthesis of prostaglandins (PG's), a defensive factor [10]. This deleterious inhibition of prostaglandin synthesis fast-tracks the activation of neutrophils and induction of local release of reactive oxygen species (ROS), and endothelin-1(ET-1) thus initiating the gastric injury [10, 11]. Among other mechanisms, oral administration of NSAIDs leads to local irritation causing back diffusion of acid into the gastric mucosa and inducing gastric damage [10].

Recent researchers have routinely applied haematological indices assessment as markers for the clinical detection of occult inflammation, inflammatory disorders, and oncogenesis [12, 13]. Monitoring of haematological changes may also complement disease-specific biochemical markers to measure the overall inflammatory status of the human body and tracking of disease progression and recovery. Alteration of white blood cells (WBC) such as leukocytes has been attributed to inflammation [14]. Derived blood count variables such as neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR), have recently been applied as complementary markers of occult inflammation in autoimmune and inflammatory disorders [12, 13]. Changes in these indices are pointers to the severity of inflammation and may predict poor outcomes in cardiovascular disease, oncologic disease, diabetes mellitus, hypertension, and auto-immune diseases [15-18]. The present study seeks to assess the alterations in haematological parameters of indomethacin-ulcerated rats treated with *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction. This study will critically enlighten on the haematological changes in the progression of gastric ulcers, as well as the possible application of haematological parameters as diagnostic markers in the pathogenesis and healing of gastric ulcers.

2. Material and Methods

2.1. Chemicals/Reagents

The gastric ulcer-inducing agent Indomethacin was obtained from Sigma-Aldrich Mo USA, Omeprazole extended-release capsules from Sanofi-aventis, Switzerland, Ethanol and ethyl acetate were obtained from JHD, China, WBC/HGB lyse reagent: Stromatolyser-WH (Sysmex, Japan). All the other reagents and chemicals used were of high analytical quality.

2.2. Plant Materials

Healthy mature fruits of *P. americana* were purchased from the Nkwo-Ugiri market and the fresh leaves of *B. pinnatum* were collected from a local garden planted at Umuodom village, Ugiri-Ike Autonomous community, Ikeduru L.G.A., Imo State, Nigeria in April 2019. The plant materials were authenticated by Prof. F. N. Mbagwu, a plant taxonomist at Imo State University, Owerri, Department of Plant Science and Biotechnology Nigeria. Plant specimens were placed in the institution herbarium and assigned voucher numbers IMSUH 0225 and IMSUH 0226 respectively.

2.3. Experimental animals

Fifty (50) healthy male Wistar albino rats (*Rattus norvegicus*) weighing 100-120 g (averaging 12 weeks old) were purchased from Animal Friend Farms, Owerri. The Wistar rats were accommodated in stainless steel cages and fed standard rat pellets and water ad libitum. The animals were acclimatized to the laboratory ambient temperature of 25±2 ˚C and relative humidity, of 55 ± 5% for 14 days before the study. The entire study was adapted to the Department of Biochemistry, Federal University of Technology, Owerri ethics committee rules with approval number BIOSC-BCH-EC-019.

2.4. Preparation of ethyl acetate fraction

The *P. americana* seed and *B. pinnatum* leaf fractions were prepared as described in the works of Asiwe et al., [19] and Asiwe et al., [20]. The fresh, healthy, sorted, and cleaned *P. americana* seed and *B. pinnatum* leaves were sliced into bits and left to air-dry at 25 ± 2 ˚C. The dried plant matter was ground to a coarse powder using a blender, Qasa grinder (QBL-8008 Pro) (China). About 250 g portion of each plant sample was extracted by maceration for 4 days using 1 litre of 80 % ethanol. The extract was filtered using Whatman No 1 filter paper and further partitioned in an ethyl acetate/water mixture, and the ethyl acetate soluble component was recovered. The fractions were dried to a slurry paste using a vacuum desiccator and designated PAS for *P. americana* seed and BPL for *B. pinnatum* leaf fraction respectively. The plant fractions were stored at 4.0 ˚C until it used for the study.

2.5. Experimental design

Ulcero-protective effect was determined as described in the work of Asiwe et al., [19] and Asiwe et al., [20]. Fifty (50) healthy Wistar albino rats were randomly assigned into ten (10) groups consisting of five (5) rats each based on their body weight (100-120 g) as shown in Table 1. Animals in group I served as normal control; the rats in groups III-X were pre-treated with 20 mgKg-1 b. wt. Omeprazole (STD), 400 mgKg-1 b. wt. PAS, 400 mgKg-1 b. wt. BPL, 400 mgKg-1 b. wt. PAS + BPL (1:1), 400 mgKg-1 b. wt. PAS + BPL (1:2), 400 mgKg-1 b. wt. PAS + BPL (1:3) respectively for 21 days (Table 1). After the 21-days treatment, the rats fasted for 24 hours and animals in groups II-X were induced for ulcers using 30 mgKg-1 b. wt. indomethacin was administered by intubation and allowed for 4 hrs before sacrificing. The groups and administration regimen are summarized in Table 1.

Table 1 Experimental design and treatment regimen

Group Number	Treatments	Group title
I	Standard rat diet and drinking water ad libitum for 21 days	Normal control (NC)
II	Indomethacin 30 mgKg-1 body weight (b. wt.)	Ulcer control (UC)
III	Omeprazole 20 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	Standard (STD)
IV	PAS 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	*P. americana* seed fraction (PAS)
V	BPL 400 mgKg-1 b.wt. + indomethacin 30 mgKg-1 b. wt.	*B. pinnatum* leaf fraction (BPL)
VI	PAS + BPL (50% : 50%) 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	PAS + BPL (1:1)
VII	PAS + BPL (33% : 67%) 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	PAS + BPL (1:2)
VII	PAS + BPL (25% : 75%) 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	PAS + BPL (1:3)
IX	PAS + BPL (67% : 33%) 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	PAS + BPL (2:1)
X	PAS + BPL (75% : 25%) 400 mgKg-1 b. wt. + indomethacin 30 mgKg-1 b. wt.	PAS + BPL (3:1)

2.6. Sacrificing and blood collection

All the rats were humanely sacrificed after 4 hours of gastric ulcer induction using dichloromethane as anesthesia. Blood samples for haematological analysis were collected by cardiac puncture and transferred to EDTA vacuum blood collection tube. The whole blood samples were transported using an ice-cold pack at a temperature of 12 ± 2 ˚C and analyzed within 30 minutes post-collection.

2.7. Determination of complete blood count (CBC)

The complete blood count was assessed using a multi-parameter automated haematology analyzer Sysmex KX-21 (Sysmex Corporation Kobe, Japan). The principle is adapted to the volumetric impedance method [21]; in which total white blood cells (WBC), red blood cells (RBC), platelets (PLT), mean corpuscular volume (MCV), mean platelet volume (MPV) are directly measured using the DC detection method; while a non-cyanide hemoglobin analysis method determines haemoglobin (HGB). Other variables such as hematocrit (HCT), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), RBC distribution width (RDW), platelet crit (PCT), and platelet distribution width (PDW) are derived variables.

2.8. Statistical Analysis

The results obtained were collated with Microsoft Excel 2010 software and expressed as mean ± standard deviation of five (n=5) determinations. Inferential statistics was performed using Statistical Package for Social Sciences (SPSS) software (version 25), and the statistical significance of values was considered at $p<0.05$ using the Turkey homogeneity of variance test.

3. Result and discussion

In the present study, the result of total white blood cell count indicates that the administration of indomethacin resulted in an inflammatory response in the rats. The total white cell count was significantly ($p<0.05$) elevated in the ulcer control group compared to normal control and ethyl acetate fraction treated groups. A similar trend was seen in the groups PAS+BPL (1:3) and PAS+BPL (3:1) (Table 2.0). Also, the differential white cell count demonstrated a toxic shift to the left with a significant proliferation of neutrophils in the UC group. However, the administration of the ethyl acetate fractions combinations PAS+BPL (1:3), PAS+BPL (1:2), and PAS+BPL (3:1) was able to normalize indomethacin-induced neutrophil leukocytosis and was comparable to the control group. Also, there was a characteristic lymphopenia and basophilia in UC, STD, PAS, and PAS+BPL (1:2) groups when compared to normal control. These characteristic neutrophilia and lymphopenia observed may be associated with the toxic changes induced by indomethacin administration which the respective ethyl acetate fractions did not ameliorate. The reduction in lymphocyte count is suggestive of a compromised immune response in the animals [22]. However, administration of BPL and other combinations in the ratios PAS+BPL (1:1), PAS+BPL (1:3), PAS+BPL (2:1), and PAS+BPL (3:1) protected against this toxic shift in leucocytes.

Table 2 Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on total and differential white blood cell count in indomethacin-induced gastric ulcer

Groups	WBC (x10³)	LYM%	MON%	NEUT%	EOS%	BAS%
NC	6.85 ± 0.59^{b}	79.70 ± 3.76^{d}	1.65 ± 0.24^{a}	12.95 ± 2.16^{a}	1.00 ± 0.08^{d}	$4.25 \pm 0.30^{a,b}$
UC	13.58 ± 0.72^{e}	40.43 ± 4.66^{a}	2.80 ± 0.14^{b}	45.40 ± 1.41^{f}	0.48 ± 0.05^{b}	11.95 ± 2.50^{e}
STD	4.75 ± 0.30^{a}	57.45 ± 6.04^{b}	3.38 ± 0.62^{b}	30.28 ± 1.56^{d}	0.25 ± 0.10^{a}	9.15 ± 0.17^{d}
PAS	5.43 ± 0.44^{a}	68.20 ± 4.57^{c}	1.78 ± 0.37^{a}	29.90 ± 3.31^{d}	0.78 ± 0.10^{c}	5.73 ± 0.53^{c}
BPL	6.68 ± 0.55b	$74.35 \pm 5.98^{c,d}$	2.80 ± 0.24^{b}	18.35 ± 1.71^{b}	0.30 ± 0.00^{a}	$4.05 \pm 0.40^{a,b}$
PAS+BPL(1:1)	8.10 ± 0.54^{c}	81.50 ± 3.82^{d}	1.40 ± 0.14^{a}	23.50 ± 4.54^{c}	0.53 ± 0.05^{b}	3.33 ± 0.25^{a}
PAS+BPL (1:2)	$7.38 \pm 0.42^{b,c}$	56.38 ± 7.02^{b}	3.05 ± 0.66^{b}	34.25 ± 4.68^{e}	0.48 ± 0.05^{b}	$5.45 \pm 0.71^{b,c}$
PAS+BPL (1:3)	15.88 ± 1.75^{f}	79.48 ± 6.39^{d}	3.08 ± 0.55^{b}	$14.75 \pm 0.66^{a,b}$	0.53 ± 0.10^{b}	3.23 ± 0.13^{a}
PAS+BPL (2:1)	6.53 ± 0.45^{b}	77.18 ± 1.47^{d}	1.95 ± 0.44^{a}	$16.25 \pm 1.52^{a,b}$	0.55 ± 0.10^{b}	$4.08 \pm 0.29^{a,b}$
PAS +BPL (3:1)	9.70 ± 0.49^{d}	$75.53 \pm 4.75^{c,d}$	2.75 ± 0.10^{b}	$16.55 \pm 2.37^{a,b}$	0.33 ± 0.05^{a}	$4.85 \pm 0.57^{b,c}$

Mean values bearing different superscript letters across columns are significantly different ($p<0.05$). LYM= lymphocytes, MON= monocytes, NEUT= neutrophils, EOS=eosinophils, BAS=basophils.

The increase in WBC count reported in this study is similar to the works of Adefisayo et al. [23]. Merchant & Modi [24] in their study reported leukocytosis in aspirin-treated rats. Furthermore, the WBC count of the indomethacin-exposed rats showed a significant increase in the activity of monocytes except for the group PAS+BPL (1:1), indicating an increased phagocytic capacity of the animals which may be ascribed to the influence of foreign compounds [25, 26]. In addition, eosinophil counts were generally increased in indomethacin-exposed rats; this may signal a decrease in the anti-inflammatory potential of these white cells.

Table 3 Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on red blood cell indices in indomethacin-induced gastric ulcer

Groups	RBC(10^6/µl)	HGB (g/dl)	HCT (%)	MCV (µm^3)	MCH (pg)	MCHC (g/dl)	RDW (µm^3)
NC	7.38± 0.37^{a,b,c}	12.33 ± 0.25^{b}	37.60 ± 2.69^{b,c}	50.98 ± 2.58^{b}	17.05 ± 0.60^{b}	33.58 ± 1.05^{a}	21.38 ± 1.11^{b,c}
UC	7.76 ± 0.21^{b,c,d}	12.65 ± 0.41^{b,c}	36.90 ± 1.58^{b,c}	47.55 ± 1.37^{c}	16.30 ± 0.35^{a,b}	34.30 ± 0.42^{a,b}	20.45 ± 0.91^{a,b,c}
STD	7.01 ± 0.21^{a}	11.08 ± 0.35^{a}	30.73 ± 1.18^{a}	43.83 ± 0.33^{a}	15.80 ± 0.00^{a}	36.05 ± 0.24^{c}	19.63 ± 0.54^{a,b}
PAS	8.16 ± 0.31^{d}	13.45 ± 0.56^{c,d}	39.50 ± 1.47^{c}	48.45 ± 1.03^{b,c}	16.48 ± 0.54^{a,b}	34.05 ± 0.44^{a,b}	20.65 ± 1.99^{a,b,c}
BPL	7.21 ± 0.56^{a,b}	12.20 ± 0.71^{b}	35.10 ± 2.10^{b}	48.75 ± 0.97^{b,c}	16.98 ± 0.33^{b}	34.75 ± 0.17^{b}	22.38 ± 0.47^{c}
PAS+BPL(1:1)	8.10 ± 0.32^{d}	12.98 ± 0.15^{b,c,d}	38.23 ± 0.59^{c}	47.20 ± 1.20^{c}	16.05 ± 0.47^{a,b}	33.98 ± 0.30^{a,b}	21.23 ± 0.70^{b,c}
PAS+BPL (1:2)	7.86 ± 0.33^{c,d}	13.00 ± 0.81^{b,c,d}	37.53 ± 1.51^{b,c}	47.78 ± 3.35^{c}	16.60 ± 1.27^{a,b}	34.70 ± 0.95^{b}	21.73 ± 2.14^{b,c}
PAS+BPL (1:3)	8.08 ± 0.18^{d}	13.58 ± 0.15^{d}	37.98 ± 1.22^{b,c}	47.10 ± 2.60^{c}	16.83 ± 0.59^{a,b}	35.78 ± 0.72^{c}	20.83 ± 1.24^{a,b,c}
PAS+BPL (2:1)	7.93 ± 0.76^{c,d}	12.68 ± 0.67^{b,c}	36.93 ± 1.89^{b,c}	46.78 ± 2.87^{c}	16.03 ± 0.84^{a,b}	34.35 ± 0.47^{a,b}	18.90 ± 1.28^{a}
PAS +BPL (3:1)	7.76 ± 0.33^{b,c,d}	12.88 ± 0.79^{b,c,d}	37.73 ± 2.62^{b,c}	48.55 ± 1.31^{b,c}	16.58 ± 0.48^{a,b}	34.18 ± 0.69^{a,b}	21.75 ± 1.72^{b,c}

Values bearing different superscript letters across columns are significantly different ($p<0.05$).

Table 4 Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on platelet indices in indomethacin-induced gastric ulcer

Groups	PLT (10^3/µl)	MPV (µm^3)	PCT (%)	PDW (%)
NC	441.25 ± 11.18^{d}	6.85 ± 0.19^{b}	0.30 ± 0.02^{e}	21.85 ± 1.50^{a}
UC	394.00 ± 10.10^{c}	6.50 ± 0.12^{a}	0.26 ± 0.02^{b,c,d}	23.63 ± 1.35^{a}
STD	390.75 ± 10.21^{c}	6.50 ± 0.00^{a}	0.25 ± 0.01^{b,c}	34.40 ± 2.07^{c}
PAS	380.25 ± 27.21^{c}	6.45 ± 0.13^{a}	0.25 ± 0.02^{b,c,d}	22.88 ± 2.17^{a}
BPL	396.75 ± 11.47^{c}	6.45 ± 0.19^{a}	0.24 ± 0.02^{b,c}	23.73 ± 1.13^{a}
PAS+BPL(1:1)	430.00 ± 12.73^{d}	6.48 ± 0.05^{a}	0.28 ± 0.01^{d,e}	23.73 ± 1.00^{a}
PAS+BPL (1:2)	384.00 ± 27.04^{c}	6.45 ± 0.19^{a}	0.26 ± 0.03^{c,d}	23.95 ± 0.10^{a}
PAS+BPL (1:3)	296.25 ± 1.50^{a}	6.95 ± 0.44^{b}	0.21 ± 0.01^{a}	37.30 ± 1.50^{d}
PAS+BPL (2:1)	339.00 ± 22.05^{b}	6.85 ± 0.29^{b}	0.23 ± 0.02^{b}	27.10 ± 1.64^{b}
PAS +BPL (3:1)	306.00 ± 20.93^{a}	6.70 ± 0.23^{a,b}	0.20 ± 0.01^{a}	27.10 ± 1.75^{b}

Values bearing different superscript letters across columns are significantly different ($p<0.05$).

Analysis of red cell indices in this study showed that indomethacin administration did not significantly alter red cell indices (Table 3.0). However, the standard caused a significant reduction of hemoglobin concentration, red cell hematocrit, MCV, and RDW and increased MCHC when compared to the NC and UC (Table 3.0). The marked decline in RBCs and Hb concentration seen among the standard treated group is indicative of microcytic anemia and anisocytosis; this alters the oxygen-carrying capacity and transport to peripheral tissues [27]. In this study, *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction and the combination did not cause marked changes in red blood cell indices.

In addition, there was a decreased thrombocyte count in groups treated with indomethacin (UC, STD, PAS, BPL, PAS + BPL (2: 1), PAS + BPL (1: 3) and PAS + BPL (3:1). However, only the groups PAS + BPL (1:1) and PAS + BPL (1:2) showed platelet count comparable to normal control. The combinations of the *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction at the different ratios resulted in a further reduction of platelet count (Table 4.0). Also, platelet variables such as MPV and PCT were not significantly altered, but the PDW was consistently increased by STD, PAS, and BPL ethyl acetate fraction combinations of PAS + BPL (1:3), PAS+BPL (2:1) and PAS + BPL (3:1) respectively compared to NC and UC.

The significant changes in the platelet variables attributed to indomethacin exposure may be indicative of megakaryocyte growth and development factor inhibition; this may alter the hemostatic capacity of the blood. Platelets are an essential part of the blood coagulation mechanism and are essential in maintaining the integrity of blood vessels by plugging the gaps in the endothelial lining. Platelets play a vital role in inflammation through activation of chemokines and inflammatory cytokines [28, 29]. They are known to interact with complement pathways as vital players in specific and non-specific immunity [30]. Under disease conditions or aseptic inflammation; post-thrombotic platelet microparticles activate adaptive immune cells leading to immunoglobulin synthesis and altered lymphocyte activities [31].

4. Conclusion

The present study demonstrated that indomethacin induction resulted in a significant increase in the total white blood cell and platelet count when compared with the control group; this was similarly seen in PAS+BPL (1:3) and PAS+BPL (3:1) combinations of PAS and BPL. Also, analysis of red blood cell variables indicates that indomethacin, *P. americana* seed, *B. pinnatum* leaf ethyl acetate fraction, and the combination did not cause marked changes in red blood cell indices. However, platelet count and platelet variables such as PDW were significantly altered, thus suggesting a compromise in the hemostatic capability of the blood.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The entire study was adapted to the Department of Biochemistry, Federal University of Technology, Owerri ethics committee rules.

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