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ORIGINAL ARTICLE

# PROTECTIVE EFFECTS OF PIROXICAM AGAINST ANTHRACENE-INDUCED HAEMATOLOGICAL AND BIOCHEMICAL ALTERATION IN FEMALE RATS

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**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**The study aimed to evaluate the protective effects of piroxicam and carboplatin against anthracene-induced haematological and biochemical alterations in female rats using randomized controlled trial. Thirty-six female rats of seven weeks old that weighed 166.25 ± 26.79 g divided into six groups of six each per group were used for the study. Each of the rats of group 1, 3, 4, 5 and 6 was administered 100 mg/kg of oral anthracene, for three weeks, except group 2, which was administered 2.5 mg/kg of water. Thereafter, each rat in groups 1 and 2 was administered water, group 3 (piroxicam low dose), group 4 (carboplatin/piroxicam), group 5 (piroxicam high dose) and group 6 (carboplatin) for a period of three weeks. Blood sample (2 ml) was collected from each rat for haematology and serum biochemistry. The rats were euthanised using 100 mg/kg of sodium pentobarbitone. Body weight, packed cell volume, erythrocyte, leucocyte and neutrophil counts were significantly decreased ($p < 0.05$), causing emaciation, anaemia, and immunodepression. Lymphocytes, monocytes, basophils, and eosinophils were significantly increased ($p < 0.05$) causing immunostimulation. Urea, creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were significantly decreased ($p < 0.05$), indicating malnutrition, reduced kidney and liver functions.**

**Keywords: amelioration; anaemia; anthracene; carboplatin; hepatitis; nephritis; piroxicam; rat**

## INTRODUCTION

Anthracene is a lung cancer-promoting polycyclic aromatic hydrocarbon (PAH) that consists of three benzene rings [1]. Exposure to anthracene in humans happens mainly through tobacco smoke [2]. It is susceptible to degradation in the presence of light [3]. Prolonged exposure causes a variety of adverse reactions [4], such as acute dermatitis, photophobia, nausea, adynamia, loss of appetite, gastroenteritis, and chronic bronchitis [5]. Anthracene has a toxic effect on the kidney and liver [6]. The toxicity effect of anthracene is dose-specific (quantitative) and exposure-specific (qualitative) [7]. It causes binucleated micronuclei, nuclear buds, and fragmented apoptotic cells [8]. However, therapeutic and toxic agents could have positive and negative effects on animals' erythrocytes [9]. Piroxicam reduces pain, inflammation, and mastitis [10], and it is excreted in urine and faeces [11]. The safe therapeutic dose of piroxicam is low, but a higher dose is toxic [12]. Therefore, there is a need for a protective agent for haematological and biochemical changes. Hence, the protective potential of piroxicam and anthracene was investigated in female rats.

## MATERIALS AND METHODS

### Animals and housing

Seven-week-old female rats weighing 166.25 ± 26.7 g were housed in metal cages and kept in the Departmental Laboratory of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria. Water and food were provided *ad libitum*. Care was provided for the animals as recommended by the Ethical Committee, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria, given the permit number (JOSTUM/CVM/ETHICS/2025/07).

### Chemicals and drugs

### Drugs

Piroxicam [Shanxi Federal Pharmaceutical Co. Ltd., China; Batch no 121103; NAFDAC no 04-6809] and carboplatin [Batch no. L01XAO2; NAFDAC no. A4-100071, German Pharmacy Company Ltd., Germany] were purchased from a pharmacy in Makurdi, Nigeria and used for the experiment.

### Preparation of anthracene solution

Approximately 20 g of anthracene powder was dissolved in 80 ml of distilled water and stirred to make a homogenous 20% (200 mg/ml) solution that gave a homogenous stable solution of pH 6.

### Preparation of piroxicam solution

Approximately 20 g of piroxicam powder from capsules was dissolved in 80 ml of distilled water and stirred to make a homogenous 20% (200 mg/ml) solution that gave a homogenous stable solution of pH 6.

### Preparation of carboplatin solution

Approximately 20 g of carboplatin powder was dissolved in 80 ml of distilled water and stirred to make a homogenous 20% (200 mg/ml) solution that gave a homogenous stable solution of pH 6.

### Selection of piroxicam, anthracene, and carboplatin therapeutic doses

The therapeutic dose selection of piroxicam was based on the LD50 of the animals determined according to the method reported by Saganuwan and Orinya [9]. An induction dose of 100 mg/kg body weight of oral anthracene administered to the experimental animals was selected based on the reported $LD_{50}$ of >5000 mg/kg body weight, whereas the experimental rats were treated according to the reported method [9–12]. Therapeutic doses of carboplatin were calculated based on the data reported by Thermo Fisher Scientific [13].

### Experimental design

A repeated measures randomized controlled trial was adopted for the study. A total of thirty-six female rats divided into six groups of six each were used for the study. Groups 1, 3, 4, 5 and 6 were administered 100 mg/kg of anthracene orally for three weeks, whereas group 2 was administered 2.5 ml of water orally.

| Group | Drugs used |
|---|---|
| 1 | Tumor control group (induced + water) |
| 2 | Normal control group (positive control) |
| 3 | Piroxicam low-dose group (2.5 mg/kg) (anthracene, piroxicam) |
| 4 | Combination therapy group (piroxicam 2.5 mg/kg + carboplatin 2.5 mg/kg) (anthracene, piroxicam, carboplatin) |
| 5 | Piroxicam high-dose group (5.0 mg/kg) (anthracene, piroxicam) |
| 6 | Carboplatin group (2.5 mg/kg) (anthracene, carboplatin) |

**Sample collection and analysis**

A pretreatment blood sample (2ml) was collected from the tail vein of each rat. After collection of blood samples on days 0, 7, and 21, groups 3, 4, 5, and 6 were treated with piroxicam (2.5 mg/kg), a combination of carboplatin (2.5 mg/kg) co-administered with piroxicam (2.5mg/kg), piroxicam (5 mg/kg) and carboplatin (2.5 mg/kg), respectively. Haematological and biochemical parameters were induced but not treated in group 1. Groups 1 and 2 were treated with 2.5 mg/kg of water [11, 14]. Animals were euthanised on day 42 using intraperitoneal injection of 100 mg/kg body weight of pentobarbital sodium [15, 16].

**Determination of hematological parameters**

Total blood volume, plasma volume, and erythrocyte volumes were determined mathematically [17, 18]. The formula is presented as follows:

$$Total\ blood\ volume\ (TBV) = Plasma\ volume\ (PV) \times \frac{100}{100 - Haematocrit}$$

Total blood volume was determined using the method of Saganuwan and Onyeyili [18]. The red blood cells and white blood cells were counted using the method of Brown [19] and Cheasebrough [20].

**Determination of renal parameters**

Serum creatinine and urea were determined using the established methods [21, 22]. Plasma creatinine was determined according to the method reported by Saganuwan [23]. Plasma creatinine was determined mathematically, using the formula given below:

$$Serum\ creatinine = \frac{Plasma\ creatinine\ (Pcr)}{1440} \times 1000$$

**Determination of hepatic enzyme parameters**

Liver enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) were determined using the established methods [24, 25, 26]. The ratio of aspartate to alanine aminotransferase was determined according to the method of Williams and Hoofnagle.

A ratio greater than 2.0 denotes alcohol liver disease, and less than 1.0 denotes chronic hepatitis and chronic cholestatic syndrome [27].

**Statistical analyses**

All the data generated were presented as mean ± standard error of mean (SEM). Two-way repeated measures analysis of variance was used to analyze the data, and a post-hoc test was used to determine significant differences among six group means [28]. Neutrophil-lymphocyte ratio was calculated according to the method reported by Zaherec [29]. The SGOT/SGPT ratio was calculated using the method reported by Cohen and Kaplan [30].

## RESULTS

**Body weights**

The effects of anthracene and piroxicam on the body weight of female rats are presented in Table 1. The weights of the animals significantly increased on day 7 and decreased on days 21, 35, and 42. However, there was no significant increase ($p > 0.05$) in the group induced with anthracene and treated with carboplatin (2.5 mg/kg), the groups treated with carboplatin (2.5 mg/kg)/piroxicam (2.5 mg/kg), the group treated with piroxicam (5 mg/kg) on the 35th and 42nd days, respectively (Table1).

**Erythrocyte parameters**

The effects of anthracene and piroxicam on the packed cell volume of female rats are presented in Table 2. There was significant decrease ($p < 0.05$) in packed cell volume (PCV) throughout the period of experimentation except for groups 4 and 5, which increased significantly on day 42. However, there was a significant increase ($p < 0.05$) in PCV in the group induced but not treated on day 21, 35, and 42, as well as groups (1, 3, and 5) induced but not treated with carboplatin (2.5mg/kg), respectively (Table 2). Erythrocyte counts decreased significantly ($p < 0.05$) throughout the period of experimentation. There was no significant increase ($p > 0.05$) in the erythrocyte counts on days 35 and 42 of the group induced with anthracene but not treated with piroxicam. However, significant increase in the erythrocyte counts ($p > 0.05$) was observed on day zero in the group neither induced nor treated with either piroxicam or carboplatin (Table 2).

Total blood volume, plasma volume, and red blood cells volumes were significantly decreased in all the groups ($p < 0.05$) except the group administered carboplatin and piroxicam (Table 2).

**Table 1. Effects of anthracene, piroxicam and carboplatin on body weight (g) of female rats**

| Experimental groups | Treatments | Days of treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 21 | 35 | 42 |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 148.00±8.09$^{bd}$ | 149.67±7.91$^{bd}$ | 166.16±7.43$^{ad}$ | 147.00±13.49$^{bd}$ | 149.67±12.91$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 143.83±8.92$^{bd}$ | 147.33±9.07$^{ad}$ | 144.00±10.04$^{bd}$ | 130.17±5.75$^{bd}$ | 134.33±4.84$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 145.50±4.35$^{bd}$ | 149.00±3.79$^{cd}$ | 145.17±9.79$^{bd}$ | 130.60±13.68$^{bd}$ | 131.40±14.15$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 146.17±4.08$^{bd}$ | 149.83±4.09$^{bd}$ | 134.50±15.92$^{bd}$ | 155.60±24.35$^{bc}$ | 166.25±26.79$^{ad}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 151.67±7.97$^{bc}$ | 154.50±7.85$^{bc}$ | 190.67±18.97$^{ac}$ | 146.80±11.33$^{bd}$ | 146.60±11.89$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 151.17±3.60$^{bd}$ | 153.50±3.72$^{ad}$ | 139.17±6.47$^{bd}$ | 132.40±8.12$^{bd}$ | 137.75±9.79$^{bd}$ |

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$)

**Leucocyte parameters**

Leucocyte counts were significantly decreased ($p < 0.05$) in all the piroxicam and anthracene-treated groups except for group 4 on days 21, 35 and 42 (Table 3). The monocyte counts were significantly ($p < 0.05$) increased in groups 1 and 2 on days 7, 21, 35, and 42 and decreased significantly in groups 3 and 6 on day 42, respectively (Table 3). Lymphocyte counts decreased significantly ($p < 0.05$) on days 7, 21, and 35 and increased significantly ($p < 0.05$) on day 42 in groups 1, 2, 3, 4, and 6, except in group 5 (Table 3). Neutrophil counts were significantly increased ($p < 0.05$) on days 7, 21, and 35, except in group 1. Meanwhile, the neutrophil counts of groups 1, 3, 4, and 6 were significantly decreased ($p < 0.05$) on day 42 except for groups 2 and 5, respectively. Neutrophil-lymphocyte ratios of the rats are presented in Table 3. The neutrophil-lymphocyte ratio was significantly increased in groups 2, and 3 (days 7, 21, 35), groups 4 and 5 (days 7, 21), and 6 (days 0, 7, 21 and 35), respectively. However, there was a significant decrease ($p < 0.05$) in neutrophil-lymphocyte ratio in all the groups except group 2 (positive control). Basophil counts were significantly increased ($p < 0.05$) on day 35 and decreased significantly ($p < 0.05$) in groups 2 and 4, they increased significantly in groups 3 and 5, respectively (Table 3). The counts of eosinophils were significantly increased in all the groups on days 7, 21, 35, and 42, except for groups 4 and 6, which decreased significantly ($p < 0.05$) (Table 3).

**Biochemical parameters**

Urea was significantly increased ($p < 0.05$) on day 21 and eventually decreased on day 42 in all the groups, respectively (Table 4). Serum creatinine was significantly increased on day 21 in the group neither induced nor treated (G 2), induced and treated with piroxicam (2.5 mg/kg), and induced and treated with carboplatin (G 6), but decreased in the group treated with piroxicam (5 mg/kg) (G 5), respectively (Table 4). Plasma creatinine was significantly decreased ($p < 0.05$) on day 42 ($p < 0.05$) in all the groups, except the group administered anthracene and water (Table 4).

Aspartate aminotransferase (AST) was significantly decreased ($p < 0.05$) in groups 2, 3, 4, and 5 and increased in groups 1 and 6 on day 21, and on day 42 (Table 5). Alanine aminotransferase (ALT) was significantly decreased ($p < 0.05$) in groups 1, 2, 3 and 5, but increased in groups 4 and 6 on day 21. However, on day 0 and 42, the values of ALT were not significantly different ($p > 0.05$). Alkaline phosphate values were significantly higher on day 21 but relatively the same on days 0 and 42 (Table 5). The significant increase ($p < 0.05$) in SGOT/SGPT ratio was observed in the groups 2 (3.95±0.69), 3 (3.41±0.62), 4 (2.30±0.27), 5 (2.85±0.23), and 6 (3.07±0.10) as compared to group 1 (1.64±0.41), respectively (Table 5).

**Table 2. Effects of anthracene, piroxicam and carboplatin on erythrocyte parameters of female rats**

| Experimental groups | Treatments | Packed cell volume (%) Days of treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 21 | 35 | 42 |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 45.83±0.5$^{bc}$ | 47.17±1.14$^{ac}$ | 45.83±1.16$^{bc}$ | 46.67±1.15$^{bd}$ | 45.17±1.60$^{bc}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 41.33±2.8$^{bd}$ | 45.83±1.74$^{ad}$ | 40.00±2.76$^{bd}$ | 40.17±3.30$^{bd}$ | 39.67±1.9$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 42.33±2.1$^{ad}$ | 38.17±1.62$^{bd}$ | 36.17±2.88$^{bd}$ | 41.80±2.65$^{bd}$ | 40.00±1.6$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 38.16±1.8$^{bd}$ | 39.33±3.17$^{bd}$ | 35.83±3.44$^{bd}$ | 41.40±1.40$^{bd}$ | 44.00±1.6$^{ad}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 41.00±2.9$^{bd}$ | 36.50±3.36$^{bd}$ | 32.17±3.55$^{bd}$ | 41.00±1.30$^{bd}$ | 43.00±1.1$^{ad}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 41.50±1.8$^{bd}$ | 41.17±2.64$^{bd}$ | 34.17±2.77$^{bd}$ | 47.00±1.47$^{ac}$ | 42.50±1.94$^{bd}$ |
| | | **Erythrocyte counts (x10$^{12}$/L)** | | | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 9.43±0.51$^{bd}$ | 7.28±0.23$^{bd}$ | 7.33±0.40$^{bd}$ | 8.98±0.29$^{bc}$ | 9.57±0.39$^{ac}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 10.04±0.45$^{ac}$ | 6.92±0.21$^{bd}$ | 6.75±0.48$^{bd}$ | 7.84±0.53$^{bd}$ | 9.03±0.31$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 9.49±0.40$^{ad}$ | 6.95±0.35$^{bd}$ | 7.44±0.71$^{bc}$ | 8.17±0.66$^{bd}$ | 8.69±0.43$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 9.03±0.31$^{ad}$ | 7.43±0.59$^{bd}$ | 6.66±0.22$^{bd}$ | 8.20±0.66$^{bd}$ | 8.77±0.56$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 9.42±0.50$^{ad}$ | 7.17±0.66$^{bd}$ | 6.76±0.59$^{bd}$ | 8.00±0.49$^{bd}$ | 8.61±0.54$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 9.49±0.53$^{ad}$ | 7.64±0.65$^{bc}$ | 6.98±0.43$^{bd}$ | 7.45±0.97$^{bd}$ | 8.20±0.75$^{bd}$ |
| | | **Blood volume (mL)** | | | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 11.84±0.64$^{bd}$ | 11.97±0.63$^{bd}$ | 13.29±0.59$^{ad}$ | 11.76±1.08$^{bd}$ | 11.97±1.03$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 11.51±0.71$^{bd}$ | 11.79±0.73$^{ad}$ | 11.52±0.80$^{bd}$ | 10.41±0.46$^{bd}$ | 10.75±0.39$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 11.64±0.35$^{bd}$ | 11.92±0.30$^{ad}$ | 11.61±0.78$^{bd}$ | 10.44±1.09$^{bd}$ | 10.51±1.13$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 11.69±0.33$^{bd}$ | 11.99±0.33$^{bd}$ | 10.76±1.33$^{bd}$ | 12.45±1.95$^{bc}$ | 13.30±2.14$^{ac}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 12.13±0.64$^{bc}$ | 12.36±0.63$^{bc}$ | 15.25±1.52$^{ac}$ | 11.74±0.91$^{bd}$ | 11.73±0.95$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 12.09±0.29$^{bd}$ | 12.28±0.29$^{ad}$ | 11.13±0.52$^{bd}$ | 10.59±0.65$^{bd}$ | 11.02±0.78$^{bd}$ |
| | | **Plasma volume (mL)** | | | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 6.41±0.64$^{bd}$ | 6.33±1.13$^{bd}$ | 7.20±1.15$^{ad}$ | 6.27±1.07$^{bd}$ | 6.57±1.01$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 6.75±0.69$^{bd}$ | 6.39±0.72$^{bd}$ | 6.91±0.78$^{cd}$ | 6.23±0.44$^{bd}$ | 6.49±0.38$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 6.71±0.34$^{bd}$ | 7.37±0.30$^{bd}$ | 7.41±0.76$^{ad}$ | 6.08±1.06$^{bd}$ | 6.31±0.71$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 7.23±0.32$^{bc}$ | 7.28±0.32$^{bd}$ | 6.91±1.29$^{bd}$ | 7.30±1.92$^{bc}$ | 7.45±2.11$^{ac}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 7.16±0.62$^{bc}$ | 7.85±0.61$^{bc}$ | 10.35±1.47$^{ac}$ | 6.93±0.99$^{bd}$ | 6.69±0.94$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 7.07±0.78$^{bd}$ | 7.23±0.29$^{bd}$ | 7.33±0.51$^{ad}$ | 5.62±0.64$^{bd}$ | 6.34±0.77$^{bd}$ |
| | | **Erythrocyte volume (x10$^{12}$/L)** | | | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 5.43±0.00$^{bc}$ | 5.64±0.50$^{bc}$ | 6.09±0.56$^{ac}$ | 5.49±0.01$^{bc}$ | 5.40±0.02$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 4.76±0.02$^{bd}$ | 5.40±0.01$^{ad}$ | 4.61±0.02$^{bd}$ | 4.18±0.02$^{bd}$ | 4.26±0.01$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 4.93±0.01$^{ad}$ | 4.55±0.00$^{bd}$ | 4.20±0.02$^{bd}$ | 4.36±0.03$^{bd}$ | 4.20±0.42$^{bc}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 4.46±0.01$^{bd}$ | 4.71±0.01$^{bd}$ | 3.85±0.04$^{bd}$ | 5.15±0.03$^{bd}$ | 5.85±0.03$^{ac}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 4.97±0.02$^{bd}$ | 4.51±0.02$^{bd}$ | 4.90±0.05$^{bd}$ | 4.81±0.08$^{bd}$ | 5.04±0.11$^{ad}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 5.02±0.01$^{bd}$ | 5.05±0.01$^{ad}$ | 3.80±0.01$^{bd}$ | 4.97±0.01$^{bc}$ | 4.68±0.01$^{bd}$ |

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row($p < 0.05$); c = significantly higher along the column($p < 0.05$); d = significantly lower along the column($p < 0.05$)

**Table 3. Effects of anthracene, piroxicam and carboplatin on differential leucocyte counts**

| Experimental groups | Treatments | Leucocyte counts ($x10^9$/L) Days of treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 21 | 35 | 42 |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 4.28±0.28$^{bd}$ | 4.35±0.26$^{ac}$ | 4.03±0.09$^{bd}$ | 3.85±0.18$^{bd}$ | 4.20±0.19$^{bc}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 4.35±0.31$^{ad}$ | 3.75±0.23$^{bd}$ | 4.02±0.08$^{bd}$ | 4.17±0.24$^{bd}$ | 3.98±0.20$^{bd}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 4.67±0.25$^{ac}$ | 3.97±0.08$^{bd}$ | 3.67±0.25$^{bd}$ | 4.02±0.16$^{bd}$ | 4.04±0.28$^{bd}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 3.68±0.21$^{bd}$ | 4.15±0.32$^{bd}$ | 4.13±0.35$^{bc}$ | 4.38±0.22$^{ac}$ | 4.05±0.34$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 3.95±0.29$^{bd}$ | 4.20±0.34$^{ad}$ | 3.55±0.19$^{bd}$ | 3.82±0.24$^{bd}$ | 3.88±0.24$^{bd}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 3.88±0.27$^{bd}$ | 3.92±0.32$^{ad}$ | 3.80±0.08$^{bd}$ | 3.77±0.31$^{bd}$ | 3.60±0.25$^{bd}$ |
| | | **Monocytes (%)** | | | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 2.00±0.41$^{bd}$ | 2.00±0.26$^{bd}$ | 2.33±0.33$^{bd}$ | 2.33±0.33$^{ad}$ | 2.33±0.33$^{ad}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 2.00±0.45$^{bd}$ | 3.00±0.57$^{bc}$ | 2.50±0.43$^{bd}$ | 2.00±0.36$^{bd}$ | 3.83±0.65$^{ac}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 3.67±0.33$^{ac}$ | 2.67±0.56$^{bd}$ | 2.17±0.31$^{bd}$ | 2.20±0.37$^{bd}$ | 2.60±0.40$^{bd}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 3.17±0.60$^{ad}$ | 2.50±0.43$^{bd}$ | 2.50±0.43$^{bd}$ | 2.20±0.58$^{bd}$ | 3.00±0.41$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 2.17±0.60$^{bd}$ | 2.83±0.40$^{bd}$ | 1.67±0.49$^{bd}$ | 3.40±0.51$^{ac}$ | 3.00±0.52$^{bd}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 3.17±0.60$^{ad}$ | 3.00±0.57$^{bc}$ | 3.00±0.36$^{bc}$ | 2.75±0.25$^{bd}$ | 2.00±0.40$^{bd}$ |
| | | **Lymphocyte counts (%)** | | | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 59.33±2.94$^{bd}$ | 60.83±1.30$^{bc}$ | 57.00±2.78$^{bc}$ | 66.00±0.96$^{ac}$ | 65.33±1.94$^{bd}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 59.83±2.57$^{ad}$ | 47.00±5.57$^{bd}$ | 40.50±3.86$^{bd}$ | 54.17±2.79$^{bd}$ | 58.17±1.07$^{bd}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 59.83±2.87$^{bd}$ | 40.00±2.82$^{bd}$ | 36.67±2.70$^{bd}$ | 52.60±1.83$^{bd}$ | 64.40±2.02$^{ad}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 65.17±2.2$^{bc}$ | 41.83±0.95$^{bd}$ | 37.67±1.08$^{bd}$ | 55.40±2.27$^{bd}$ | 68.75±2.17$^{ac}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 64.83±1.9$^{ad}$ | 37.33±3.18$^{bd}$ | 43.00±4.53$^{bd}$ | 54.80±1.96$^{bd}$ | 62.00±3.11$^{bd}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 33.67±4.0$^{bd}$ | 36.17±1.35$^{bd}$ | 45.17±7.17$^{bd}$ | 52.25±2.09$^{bd}$ | 68.50±1.32$^{ad}$ |
| | | **Neutrophil counts (%)** | | | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 37.00±3.04$^{bc}$ | 34.67±1.28$^{bd}$ | 37.33±2.36$^{ad}$ | 29.33±1.14$^{bd}$ | 30.33±1.76$^{bd}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 34.67±3.12$^{bd}$ | 48.17±5.72$^{bd}$ | 54.83±3.92$^{ad}$ | 41.17±2.33$^{bd}$ | 36.17±0.91$^{bc}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 36.00±3.02$^{bd}$ | 55.50±2.94$^{bd}$ | 58.33±2.55$^{ac}$ | 42.60±2.38$^{bc}$ | 30.80±2.01$^{bd}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 30.67±1.65$^{bd}$ | 52.67±1.17$^{bd}$ | 57.33±1.63$^{ad}$ | 39.80±2.52$^{bd}$ | 27.00±1.96$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 31.35±2.01$^{bd}$ | 56.67±2.68$^{ad}$ | 53.00±4.38$^{bd}$ | 39.80±1.56$^{bd}$ | 32.20±3.08$^{bd}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 33.67±4.08$^{bd}$ | 58.50±1.34$^{ac}$ | 49.67±7.37$^{bd}$ | 42.25±1.65$^{bd}$ | 28.50±1.55$^{bd}$ |
| | | **Neutrophil-lymphocyte ratio** | | | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 0.62±0.42$^{ad}$ | 0.57±0.40$^{bd}$ | 0.65±0.35$^{ad}$ | 0.44±0.20$^{bc}$ | 0.46±0.23$^{bd}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 0.58±0.20$^{bd}$ | 1.02±0.42$^{ac}$ | 1.35±0.41$^{ac}$ | 0.76±0.34$^{bc}$ | 0.62±0.35$^{bc}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 0.60±0.18$^{bd}$ | 1.39±0.43$^{ac}$ | 1.59±0.39$^{ac}$ | 0.81±0.22$^{bc}$ | 0.48±0.17$^{bd}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 0.47±0.13$^{bd}$ | 1.26±0.42$^{ac}$ | 1.52±0.25$^{ac}$ | 0.72±0.19$^{bd}$ | 0.39±0.15$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 0.48±0.18$^{bd}$ | 1.52±0.34$^{ac}$ | 1.23±0.39$^{ac}$ | 0.73±0.32$^{bd}$ | 0.52±0.17$^{bd}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 1.00±0.17$^{ac}$ | 1.62±0.41$^{ac}$ | 1.10±0.17$^{ac}$ | 0.81±0.32$^{bc}$ | 0.42±0.20$^{bd}$ |
| | | | | **Basophils (%)** | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 1.17±0.40$^{bc}$ | 1.50±0.43$^{bd}$ | 1.83±0.31$^{ac}$ | 1.17±0.31$^{bd}$ | 1.17±0.40$^{bd}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 1.17±0.16$^{bc}$ | 1.33±0.33$^{bd}$ | 1.33±0.33$^{bd}$ | 1.67±0.49$^{ad}$ | 1.00±0.36$^{bd}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 0.16±0.16$^{bd}$ | 1.17±0.31$^{bd}$ | 1.83±0.31$^{ac}$ | 1.40±0.40$^{bd}$ | 1.20±0.37$^{bd}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 0.50±0.22$^{bd}$ | 1.67±0.21$^{bc}$ | 1.83±0.49$^{ac}$ | 1.60±0.24$^{bd}$ | 0.75±0.47$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 1.00±0.36$^{bd}$ | 1.33±0.42$^{bd}$ | 1.50±0.43$^{bd}$ | 1.20±0.20$^{bd}$ | 1.80±0.37$^{ac}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 0.66±0.49$^{bd}$ | 1.33±0.33$^{bd}$ | 1.33±0.21$^{bd}$ | 1.75±0.85$^{ac}$ | 0.75±0.25$^{bd}$ |
| | | | | **Eosinophil counts (%)** | | |
| **1.** | Induced not treated Water (2.5ml) (Negative control) | 0.50±0.22$^{bd}$ | 1.00±0.25$^{bd}$ | 1.50±0.34$^{ac}$ | 1.17±0.31$^{bd}$ | 0.83±0.31$^{bd}$ |
| **2.** | Neither induced nor treated Water (2.5ml) (Positive control) | 0.66±0.21$^{bc}$ | 0.83±0.31$^{bd}$ | 1.00±0.36$^{ad}$ | 1.00±0.45$^{ad}$ | 0.83±0.16$^{bd}$ |
| **3.** | Induced and treated Piroxicam (2.5 mg/kg) | 0.33±0.21$^{bd}$ | 0.66±0.21$^{bd}$ | 1.00±0.36$^{bd}$ | 1.20±0.37$^{ac}$ | 1.00±0.32$^{bc}$ |
| **4.** | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 0.50±0.34$^{bd}$ | 1.16±0.31$^{bc}$ | 1.17±0.63$^{ad}$ | 1.00±0.32$^{bd}$ | 0.50±0.28$^{bd}$ |
| **5.** | Induced and treated Piroxicam (5 mg/kg) | 0.66±0.21$^{bc}$ | 0.83±0.40$^{bd}$ | 0.83±0.40$^{bd}$ | 0.80±0.37$^{bd}$ | 1.00±0.45$^{ac}$ |
| **6.** | Induced and treated Carboplatin (2.5 mg/kg) | 0.33±0.21$^{bd}$ | 1.00±0.26$^{ad}$ | 0.83±0.30$^{bd}$ | 1.00±0.41$^{ad}$ | 0.25±0.25$^{bd}$ |

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$); neutrophil-lymphocyte ratio = 1–2 (normal); >3.0–0.7 (pathological); 2.3–3.0 (cancer atherosclerosis, infection, inflammation, stress, psychiatric disorder); 2.5–5.0 (solid tumor)

**Table 4. Effects of anthracene, piroxicam and carboplatin on kidney function parameters of female rats**

| Experimental groups | Treatments | Urea (mmol/L) Days of treatment 0 | 21 | 42 |
|---|---|---|---|---|
| 1. | Induced not treated Water (2.5ml) (Negative control) | 6.19±0.32$^{bc}$ | 12.45±0.15$^{ad}$ | 6.19±0.32$^{bc}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 3.12±1.46$^{bd}$ | 12.60±0.40$^{ad}$ | 3.22±1.46$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 3.23±0.48$^{bd}$ | 11.50±1.40$^{ad}$ | 3.23±0.48$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 5.59±0.02$^{bd}$ | 11.15±0.45$^{ad}$ | 5.59±0.01$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 5.87±0.81$^{bc}$ | 14.10±0.10$^{ac}$ | 5.87±0.18$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 4.19±0.89$^{bd}$ | 10.03±0.47$^{ad}$ | 4.19±0.89$^{bd}$ |
| | | **Serum creatinine (µmol/L)** | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 99.50±9.00$^{ac}$ | 66.85±19.05$^{bd}$ | 99.45±9.05$^{ac}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 43.25±3.98$^{bd}$ | 77.60±0.50$^{ad}$ | 58.25±2.49$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 50.65±2.35$^{bd}$ | 86.70±3.90$^{ad}$ | 50.65±2.35$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 77.50±1.50$^{bd}$ | 81.15±6.15$^{ad}$ | 77.50±1.50$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 91.95±16.85$^{ad}$ | 88.50±1.31$^{bc}$ | 91.95±16.85$^{ad}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 57.65±8.35$^{bd}$ | 73.25±9.35$^{ad}$ | 57.65±8.35$^{bd}$ |
| | | **Plasma creatinine (µmol/L)** | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 143.28±12.96$^{ac}$ | 96.25±27.43$^{bd}$ | 143.23±13.03$^{ac}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 62.28±5.73$^{bd}$ | 111.74±0.72$^{ad}$ | 83.88±3.59$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 72.94±3.38$^{bd}$ | 124.85±5.62$^{ad}$ | 72.94±3.38$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 111.60±2.16$^{bd}$ | 116.86±8.86$^{ad}$ | 111.60±2.16$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 132.41±24.26$^{ad}$ | 127.44±1.89$^{bc}$ | 132.41±24.26$^{ad}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 83.02±12.02$^{bd}$ | 105.48±13.64$^{ad}$ | 83.02±12.02$^{bd}$ |

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$)

**Table 5. Effects of anthracene, piroxicam and carboplatin on liver function parameters of female rats**

| Experimental groups | Treatments | Aspartate aminotransferase U/L (SGOT) | | |
|---|---|---|---|---|
| | | Days of treatment | | |
| | | 0 | 21 | 42 |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 164.10±0.60$^{bd}$ | 183.80±2.00$^{ad}$ | 164.10±0.60$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 190.10±5.50$^{ad}$ | 42.60±1.49$^{bd}$ | 190.10±5.50$^{ad}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 293.65±2.99$^{ad}$ | 114.70±1.81$^{bd}$ | 293.65±2.99$^{ad}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 230.25±2.16$^{ad}$ | 176.55±2.44$^{bd}$ | 230.25±2.16$^{ad}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 318.40±3.26$^{ac}$ | 104.85±7.28$^{bd}$ | 318.40±3.26$^{ac}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 252.75±4.37$^{bd}$ | 266.75±9.95$^{ac}$ | 252.75±4.37$^{bd}$ |
| | | **Alanine aminotransferase U/L (SGPT)** | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 100.30±0.60$^{ad}$ | 77.45±1.05$^{bd}$ | 100.30±0.60$^{ad}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 190.10±5.50$^{ac}$ | 28.40±2.38$^{bd}$ | 48.15±28.05$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 86.20±1.96$^{ad}$ | 64.62±1.26$^{bd}$ | 86.20±1.96$^{ad}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 100.20±3.27$^{bd}$ | 103.05±4.35$^{ad}$ | 100.20±3.27$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 111.80±5.80$^{ad}$ | 43.20±4.60$^{bd}$ | 111.80±5.80$^{ac}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 82.25±17.15$^{bd}$ | 121.90±5.80$^{ac}$ | 82.25±17.15$^{bd}$ |
| | | **SGOT-SGPT ratio** | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 1.64±0.41$^{bd}$ | 2.37±0.78$^{ac}$ | 1.64±0.41$^{bd}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 1.90±0.41$^{bd}$ | 1.50±0.26$^{bd}$ | 3.95±0.69$^{ac}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 3.41±0.62$^{ac}$ | 1.77±0.59$^{bd}$ | 3.41±0.62$^{ad}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 2.30±0.27$^{ad}$ | 1.71±0.23$^{bd}$ | 2.30±0.27$^{ad}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 2.85±0.23$^{ad}$ | 2.43±0.65$^{bc}$ | 2.85±0.23$^{ac}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 3.07±0.10$^{ad}$ | 2.19±0.29$^{bd}$ | 3.07±0.10$^{ad}$ |
| | | **Alkaline phosphatase U/L (ALP)** | | |
| 1. | Induced not treated Water (2.5ml) (Negative control) | 9.25±0.45$^{ac}$ | 25.85±8.15$^{bc}$ | 9.25±0.45$^{ac}$ |
| 2. | Neither induced nor treated Water (2.5ml) (Positive control) | 6.90±4.60$^{bd}$ | 17.55±0.15$^{ac}$ | 6.90±4.60$^{bd}$ |
| 3. | Induced and treated Piroxicam (2.5 mg/kg) | 6.00±0.50$^{bd}$ | 9.45±8.25$^{ad}$ | 6.00±0.50$^{bd}$ |
| 4. | Induced and treated Carboplatin (2.5 mg/kg) Piroxicam (2.5 mg/kg) | 7.40±1.10$^{bd}$ | 9.75±4.55$^{ad}$ | 7.40±1.10$^{bd}$ |
| 5. | Induced and treated Piroxicam (5 mg/kg) | 5.50±0.30$^{bd}$ | 15.35±0.05$^{ad}$ | 5.50±0.30$^{bd}$ |
| 6. | Induced and treated Carboplatin (2.5 mg/kg) | 7.05±1.25$^{bd}$ | 12.95±0.45$^{ad}$ | 7.05±1.25$^{bd}$ |

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$); SGOT/SGPT ratio = >1.0 (chronic liver inflammation; <1.0 hepatitis, non-alcoholic fatty disease, bile duct problem); 1 (drug induced liver inflammatory)

## DISCUSSION

### Effects of anthracene, piroxicam, and carboplatin on weight gain

The increased weight gain observed on day 35, and day 21 in group 4 (2.5 mg/kg carboplatin/2.5 mg/kg piroxicam) and the weight loss observed in group 5 (5 mg/kg piroxicam) show that a combination of carboplatin/piroxicam has weight-increasing potential and piroxicam alone has weight reducing potential. However, increased weight gain observed in group 4 shows that carboplatin could improve weight when co-administered with piroxicam for a period of 3 weeks [31, 32, 33].

### Effects of anthracene, carboplatin, and piroxicam on haematological parameters

The decreased PCV caused by anthracene and restored by carboplatin and piroxicam shows that both can be used to ameliorate anthracene-induced haemolysis in rats [31, 34]. However, the decreased erythrocyte counts in all the anthracene- and piroxicam-treated groups show that erythrocyte counts are independent of the packed cell volume. Hence, piroxicam and anthracene can cause anaemia [35] that could be exacerbated by some anti-cancer drugs [36], as observed in the group administered carboplatin. Also, it was reported that decreased red blood cell width distributions (RWDs) as observed in the present study is a strong index of survival in cancer patients [37]. The leucopenia observed in the present study is in disagreement with the findings that leucocytosis is a prognostic marker of lung cancer [37–39]. Also, the leucocyte count is independent of physical activity in lung cancer patients [40]. This further suggested that leukocytosis is an important biomarker for increased risk of lung damage [41]. However, tumour-related leukocytosis is related importantly to non-small cell carcinoma, and it is an ominous prognostic marker [39]. Leucocytosis could be attributed to monocytosis and lymphocytosis observed in the present study. The observed neutrophilia and neutropenia in the treated group agree with the report indicating that neutrophils could be used as a biomarker of cancer detection. Tumour-associated neutrophilias (TANs) could be controlled by tumour microenvironment and aid in tumour progression. Hence, TANs could be harmful and beneficial [42]. Certainly, neutrophilia is a negative prognostic factor in lung cancer patients, but other white blood cells do not affect patient's survival [43]. Basophilia and eosinophilia observed in the present study show that they could be used as prognostic biomarkers for piroxicam treatment. Whereas basopenia and eosinopenia could be prognostic biomarkers for carboplatin treatment [44, 45].

### Effects of anthracene, carboplatin, and piroxicam on biochemical parameters

The administration of piroxicam may cause a significant increase in the levels of ALT and AST [31, 46], is characterized by a remarkable cellular infiltration of hepatic tissue by certain chemicals [47, 48]. The decreased alanine aminotransferase and alkaline phosphatase observed in the present study show that anthracene and carboplatin could inhibit the hepatic enzymes. However, 5 mg/kg of piroxicam restored the enzymes to their normal values. Piroxicam could modulate humoral immune system, induce liver enzymes, enhance renal function, and reduce the allometric parameter of the liver [49]. Meanwhile, delayed absorption and distribution of piroxicam could provide the desired therapeutic effect with less toxicity [50], suggesting a strong correlation between the two enzymes in liver problems [51]. The aspartate alanine amino transferase level greater than 2.0 in the present study indicates hepatic inflammation. This effect may be due to keto-enol tautomerism of piroxicam. The enol functional group could have been responsible for the rise in the ratio [52]. Some tautomers cause damage to tissues [53], indicating the importance of toxicity studies of drugs in animals for identification of potential hazards [54]. Hydroxyl and oxygen functional groups are responsible for tissue damage as revealed by liver enzyme markers [55], suggesting that the knowledge of therapeutics could be highly beneficial in anthracene poisoning [56]. The restoration of urea and creatinine to normal after sharp increases shows that a carboplatin dose could be administered based on normal renal function [57]. Hence, body surface area, body weight, creatinine clearance, glomerular filtration rate, creatinine half-life, and age are necessary parameters for assessing renotoxicity that may be caused by carboplatin and piroxicam [23]. Lower neutrocyte-lymphocyte ratio (NLR) is associated with reduced systemic inflammation and longer survival [58]. The increased SGOT/SGPT ratio observed in the present study shows that the livers of the group 2–6 rats were inflamed, indicating that the SGOT/SGPT ratio is a marker of liver damage [30].

### Mechanisms of action of anthracene, carboplatin, and piroxicam

The metabolite of anthracene (2-methly-1-benzofurann-3-carbaldelyde) increases oxygen affinity of haemoglobin to prevent polymerization of haemoglobin thereby inhibiting red blood cell sickling, leading to metabolic instability caused by the aldehyde functional group [59, 60]. Anthracene could be converted to anthraquinone [61]. Presence of aldehyde functional and ketone functional groups in the anthracene metabolites suggest that anthracene could be used to induce cell damage [62]. Hence, it could be an inducer and an inhibitor of biological activities [63]. Carboplatin, a derivative of cisplatin, causes DNA damage by forming adducts with platinum, leading to inhibition of replication, transcription, and cell death [64]. Piroxicam inhibits tissue cyclooxygenases 1 and 2 (COX-1 and -2) resulting in reduced synthesis of pro-inflammatory prostaglandins that mediate inflammation and pain [65]. Piroxicam also inhibits N-methyl-D-aspartate receptor [66], suggesting that piroxicam could inhibit aspartate aminotransferase enzyme. Aspartate, glutamate, D-alanine, asparagine and L-alanine are derived from an intermediate of citric acid cycle [67].

### Study limitations and recommendations for future research

The species of animals, sex, age, and the research environment are major limitations, connoting that general laboratory practice (GLP) and standard operating procedure (SOP) could be responsible for the variations in the results. Methods of the haematological and biochemical analyses of the parameters, and the data generated, could contribute greatly to the differences in the reported results. Meanwhile, we recommend biochemical, molecular, cellular, and physiological elucidation of the derangements in the parameters. Hence, further studies are necessary aimed at elucidating pathophysiology, biochemical pharmacology, and biochemical toxicology of anthracene, carboplation, and piroxicam and their mechanistic interplay. Relationship between obesity, anaemia, polycythemia, immune stimulation, and transminitis should be studied. Hyperuremia, hypecreatinaemia and hyperphosphataemia associated with impaired kidneys, particularly, chronic kidney disease, need to be studied in relation to anthracene-carboplatin-piroxicam interplay.

## CONCLUSION

Anaemia, leucopenia, monocytopenia, lymphopenia, neutrophilia, basophilia, eosinopenia, increased urea, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphate were observed, suggesting imunomodulatory potentials of anthracene, carboplatin, and piroxicam.

### Ethical Statement

All the rats used for the study were handled according to the guide principles of the Animal Ethics Committee of Joseph Sarwuan Tarka University Makurdi given the permit number (JOSTUM/CVM/ETHICS/2025/07).

### Conflict of Interest

The authors have no conflict of interest to disclose.

### Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

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### Authors' Contributions

SAS designed the study and analyzed the data. AMU carried out the study and collected the data. SAS, AMU, AMV, and TA wrote, proofread and approved the manuscript.

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No generative artificial intelligence (AI) and AI-assisted technologies were used in writing the manuscript.

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