

# Constituents of Pomegranate Peel Extract, and its Ameliorative Effect Against the Toxicity of Vancomycin on the Hematological Parameters of Male Albino Rats

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**Abstract:** Vancomycin (VCM) is a widely used antibiotic known for its efficacy against severe bacterial infections, but its administration is often associated with hematological toxicity. This study investigates the protective effects of pomegranate peel extract (PPE) against VCM-induced hematological alterations in male Albino rats. The GC-MS analysis of PPE revealed 22 bioactive components, with major constituents being (E)-9-Octadecenoic acid methyl ester, methyl 9-cis,11-trans-octadecadienoate, and 9-octadecenoic acid (E)-, collectively contributing to its potent antioxidant and anti-inflammatory properties. Thirty-two adult male Albino rats were divided into four groups, (n=8 each): control, PPE alone, VCM alone, and VCM with PPE. The control group was fed the basal diet; the VCM-injected group received VCM (443.6 mg/kg B.W.) every other day for two weeks; the PPE-treated group which was given the basal diet, and a daily dose of PPE (100 mg/kg B.W.) orally for two weeks; and the VCM combined with PPE- group administered the basal diet, and both VCM (every other day) and PPE (daily). After 14 days, hematological parameters, including RBC count, Hb content, Hct %, MCV, WBC count, and differential leukocyte counts, were evaluated. VCM administration significantly reduced RBC count, Hb content, and Hct %, while increasing MCV and altering leukocyte profiles, indicative of hematological toxicity. PPE co-administration effectively ameliorated these adverse effects, restoring RBC indices and reducing inflammation as evidenced by normalized neutrophil-to-lymphocyte ratios. The findings demonstrate that PPE, rich in bioactive fatty acids and antioxidants, mitigates VCM-induced hematological toxicity. These results suggest that PPE has the potential as a protective agent in clinical settings to counteract the adverse hematological effects of VCM therapy. Further studies are warranted to elucidate the underlying mechanisms and to explore the clinical applications of PPE in managing antibiotic-induced hematological disorders.

**Keywords:** Vancomycin, Pomegranate peel extract, *Punica granatum*, Blood parameters, Erythrogram, Leukogram, Hematopoietic toxicity.

## 1. Introduction

Vancomycin (VCM) is a potent, narrow-spectrum bactericidal antibiotic belonging to the glycopeptide class of antibiotics. Its nomenclature was derived from the word “vanquish”, reflecting its strong antibacterial activity against Gram-positive strains, specifically, *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aur[1]jeus* (MRSA) which can trigger a variety of organspecific infections [1, 2]. Most importantly, VCM considered a last resort treatment for serious MRSA infections, especially when other antibiotics have failed [3]. However, like most antibiotics, VCM has been proven to cause side effects that can limit its clinical use [4]. It may induce some alterations in the haematological parameters since it has been reported to cause haemolytic anemia [5].

One approach to reducing the adverse effects of VCM in therapeutic applications is the use of natural compounds with

beneficial biological activities. In this aspect, there has been a growing interest in herbal medicine [6].

Pomegranates (*Punica granatum L.*) are a widely consumed fruit known for their taste and health benefits. Native to the Middle East, their cultivation has expanded to diverse geographical areas throughout the globe, leading to significant genetic and phytochemical diversity [7]. Traditionally, pomegranate fruits have been utilized in folk medicine for thousands of years. The pomegranate fruit comprises three main parts: peel, aril, and seeds [8].

Pomegranate peel (PP), is a significant byproduct of pomegranate processing accounting for nearly half of the fruit by weight [9]. Interestingly, the inedible portions of the pomegranate fruit, such as the peel and membrane layers, have higher concentrations of phytochemicals than the edible flesh pulp, making them a rich source of bioactive compounds [10]. PP has been reported to own anti-anemic and cardioprotective

properties [11]. Also, the alcoholic extract of the PP has been shown to significantly increase RBC count, Hb levels, and Hct in male Albino rats treated with erythromycin [12].

Given these properties, our study was designed to find out the role of pomegranate peel water extract (PPE) supplementation in alleviating haematological changes induced by VCM in mature male albino rats.

## 2. Materials and methods

### 2.1. Tested drug and plant peel extract:

Edicin® 500 mg (vancomycin 500 mg; M.O.H. Reg. No.: 31715/2016) is a white crystalline lyophilized powder for solution for IV infusions manufactured by Global Pharmaceutical Industries (GPI), Cairo, Egypt. Each ampoule contains 512.57 mg vancomycin hydrochloride, equivalent to 500 mg of vancomycin, which was dissolved in distilled water.

For the preparation of the pomegranate peel extract (PPE), fresh pomegranate peels were carefully washed under running water and cleaned. The peels were collected as byproducts from a well-known juice stores in Sohag Governorate. The individuals involved were instructed to keep the peels refrigerated until they were received.

### 2.2. Preparation of the PPE:

Two kilograms of the washed and cleaned pomegranate peels were placed in a stainless-steel cooking pot with one liter of bi-distilled water and heated for 7 hours without boiling. After cooling, the mixture was passed through a mesh sieve to obtain the extract, was then, purified using Whatman No. 1 filter paper to remove impurities. The net yield of the extract was approximately 800 ml, and it was stored in the refrigerator at 4°C until use [13].

### 2.3. Gas Chromatography-Mass Spectrometer (GC-MS) Analysis:

The chemical composition of PPE was analyzed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness) at the Lab of Functional Foods, Department of Dairy Sciences and Technology, Faculty of Agriculture, El Shatby Campus, Alexandria University, Alexandria, Egypt. The column oven temperature program was adjusted according to the method described by El Bohi *et al.* [4]. The components were identified by comparing the mass spectra of the components to those in the National Institute of Standards and Technology (NIST14) mass spectral database and the Wiley Registry mass spectral libraries (WILEY 09).

### 2.4. Animals and experimental design:

Thirty-two healthy male adult Albino rats, aged 3-4 months with an average initial body weight of approximately 176±10 gm, were purchased from the Animal House, Faculty of Science, Sohag University, Sohag, Egypt. During the experiment, the rats were housed in stainless-steel cages in an air-conditioned room at a temperature (25°C±2°C) with a 12-hour light/12-hour dark cycles. Animals were provided with feed and fresh water *ad libitum*.

After a week of adaptation, the animals were arbitrarily allocated into four groups, (n=8 animals/group), as follows: Group 1: Control group provided with water and food *ad libitum*. Group 2: VCM-injected group, injected with a daily dose of VCM (444 mg/kg B.W.) intraperitoneally (IP) every other day for 2 weeks [4]. Group 3: PPE-treated group, received PPE orally at a dose of 100 mg/kg B.W. daily for 2 weeks [4]. Group 4: VCM+PPE-treated group, injected with a daily dose of VCM every other day for 2 weeks, in addition to receiving PPE orally at a dose of 100 mg/kg B.W. daily for 2 weeks [4]. The experimental protocol was approved by the Ethical Committee of Sohag University (CSRE-2-24).

### 2.5. Collection of blood samples:

Twenty-four hours following the last VCM injection, animals were sacrificed and whole blood samples were collected from the heart using a sterile syringe into a sterile tube containing an anti-coagulant (EDTA). These blood samples were immediately used for measuring the complete blood count (CBC), including red blood corpuscles (RBCs), hemoglobin (Hb), hematocrit (Hct %), mean red blood cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTs), white blood cells (WBCs) and differential leukocytes (neutrophils, lymphocytes, and monocytes) using an automated hematological analyzer (Celltac, Japan) at Target Clinical lab, Akmim, Sohag, Egypt.

### 2.6. Statistical analysis:

The normality and homogeneity of the data were evaluated using the Shapiro-Wilk W test and the homogeneity of variances test, respectively. To determine statistical differences among the experimental groups, a one-way analysis of variance (ANOVA) was performed. Statistical analysis was carried out by the SPSS 21.0 computer program (IBM Inc, Armonk, NY)[14]. Duncan's multiple comparisons post hoc test was done to compare the mean values of the different groups. Statistical significance was assumed at a *P-value* of less than 0.05 ( $P < 0.05$ ). The results were presented as mean values and standard error of the mean (Mean ± SEM).

## 3. Results:

### 3.1. GC-MS Analysis:

The GC-MS profile of PPE showed that the extract contained 22 components with varying retention times (RT), representing 100% of the total extract composition. According to the peak areas (%), the major compounds identified, arranged in order from the highest to the lowest ratio, were (E)-9-Octadecenoic acid methyl ester (methyl oleate), methyl 9-cis,11-trans-octadecadienoate (methyl linoleate), 9-octadecenoic acid, (E)- (oleic acid), with peak areas (%) of 40.94, 20.7, and 11.19, respectively (Table 1, Fig. 1).

The chromatograph also displayed several other peaks with lower heights, the presence of other compounds in smaller amounts. These minor components, although present in lesser quantities, may contribute significantly to the biological activities of the extract.

### 3.2. Hematological parameters:

The erythrogram illustrated significant changes in the hematological parameters among the different groups studied. Compared to the control and PPE-administered groups, the mean values of RBC count, Hb content, and Hct were significantly decreased in the VCM-treated group. Conversely, the RBC indices of the PPE co-administrated group demonstrated statistically significant improvements in these parameters compared to the VCM-treated group (**Table 2**). Furthermore, the results exhibited a significant increase in the mean value of MCV in VCM-treated rats, compared to the control and PPE-administrated groups.

However, the PPE co-administrated group showed a

significant recovery in the MCV, relative to the VCM-treated group (**Table 2**). PLT counts did not show significant differences across all treated groups (**Table 3**).

The leukogram analysis showed that the mean value of total WBC count was highest in the VCM-treated rats relative to the control, although this increase was mild and statistically non-significant. The differential leukocyte count indicated that neutrophil counts were significantly elevated in both VCM-treated and VCM+PPE groups, while, lymphocyte counts were significantly lowered in these two groups compared to the other groups in the study (**Table 4**). The neutrophil-lymphocyte ratio (NLR) was highly significant in the VCM-treated group, and the VCM+PPE group (**Table 5**).

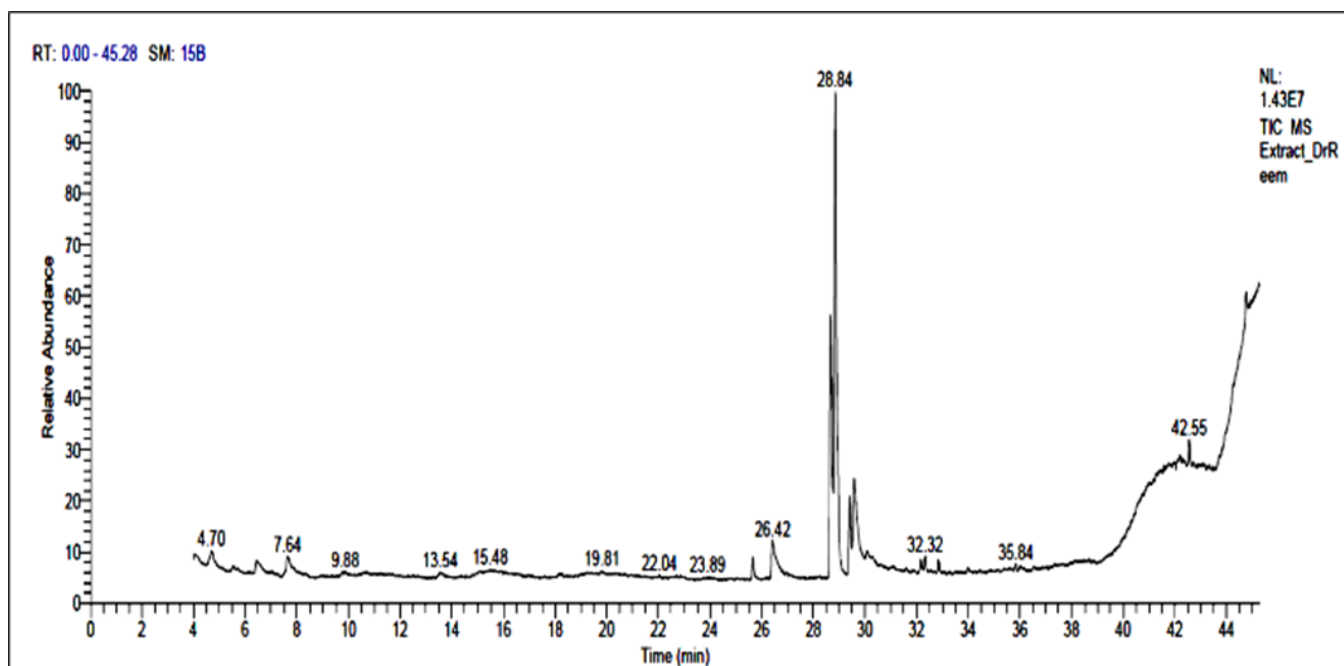


Fig. 1: GC-MS chromatogram of PPE aqueous extract.

## 4. Discussion

Haematological indices are critical in assessing general health status, detecting a variety of disorders, distinguishing anaemias, and quantifying many blood components and features. In many health conditions, specific symptoms of illness may not be apparent, and changes in the CBC may be the sole finding. Consequently, data regarding haematological parameters are exceptionally important [15].

In the current study, VCM significantly lowered RBC count, Hb content, and Hct %, consistent with previous studies. This finding aligns with Siddiqui *et al.* [5], who reported a case of a 75-year-old patient suffered from hemolytic anemia within a week of starting VCM treatment for a joint infection. The-Hb levels significantly dropped after commencing VCM, despite the patient previous-antibiotics treatments for chronic knee infections and-improved after stopping VCM. Similarly, Kannan & Raj [16] published a case report of a 56-years-old man commenced VCM treatment at a dose of (1 g/12 hr) for an open ulcer, showing lower Hb, and Hct on day 4, with

moderate hematuria. Sadeghi *et al.* [17] also found that rats injected with VCM (200 mg/kg i.p. every 12 hr for 7 days) suffered from hematuria. On the contrary, Uhuo *et al.* [18] reported no significant changes in hematological parameters in rats receiving VCM injections twice a day for 14 days (200 mg/kg B.W. Balkrishna *et al.* [19] observed similar results using 200 mg/kg VCM injections twice a day for 7 consecutive days in the morning and 150 mg/kg at night, every 12 hr.

The moderate reductions in RBCs, Hb, and Hct % observed in this study may be due to VCM's oxidative properties, stimulating free radical production. Oxidative stress is a known mechanism through which VCM induces toxic effects on the liver [20], kidneys [21], and testis [22].

As oxygen carriers, RBCs are constantly facing high levels of oxygen tension. Erythrocytes are the major targets for reactive oxygen species (ROS) and oxidative stress in circulation, and it is the primary cause of RBCs aging which

impairing their biomechanics and oxygen delivery ability. So, they are being protected by antioxidants to complete their lifespan of more than 100 days [23]. The oxidative stress triggers irreversible damage to RBCs because they lack cell organelles and cannot repair damaged components, lowering their antioxidant capacity and causing them to be degraded by

hemolysis, and eliminated from circulation [24]. This can contribute to anemia through increased RBC breakdown [25]. Berger *et al.* [26] explained that some hemolytic anemias are drug-induced, including non-immune DIHA, which occurs when RBCs encounter drugs causing oxidative damage [27].

**Table 1:** Bioactive phytochemical constituents assigned in PPE by GC-MS analysis:

No.	RT (min)	Compound Name	Area (%)	M. F.	M. W. (g/mole)
1	4.04	D-Fructose, diethyl mercaptal, pentaacetate	1.11	C <sub>20</sub> H <sub>32</sub> O <sub>10</sub> S <sub>2</sub>	496
2	4.70	Dodecanoic acid, 2,3- bis (acetyloxy) propyl ester	2.23	C <sub>19</sub> H <sub>34</sub> O <sub>6</sub>	358
3	5.54	2(3h)-Furanone, 5-Heptyldihydro	0.60	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	184
4	6.44	Methyl 6-oxoheptanoate "Heptanoic acid"	2.11	C <sub>6</sub> H <sub>14</sub> O <sub>3</sub>	158
5	7.65	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	3.00	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144
6	13.56	9,10-secochola-5,7,10(19) -triene-3,24-diol, (3á, 5Z,7E)-	0.61	C <sub>24</sub> H <sub>38</sub> O <sub>2</sub>	358
7	25.65	Hexadecanoic acid, methyl ester	1.60	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
8	26.42	Hexadecanoic acid	4.62	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
9	28.66	Methyl 9-cis,11-trans-octadecadienoate	20.78	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
10	28.84	(E)-9-Octadecenoic acid methyl ester	40.94	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
11	29.41	Octadecanoic acid, methyl ester	4.75	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
12	29.57	9-Octadecenoic acid, (E)-	11.19	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
13	30.09	9-octadecenoic acid (z)-	0.84	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
14	30.19	Digitoxin	0.27	C <sub>41</sub> H <sub>64</sub> O <sub>13</sub>	764
15	30.27	9,10-secochola-5,7,10 (19) -trien-24-al, 3-hydroxy-, (3á,5z,7e) -	0.76	C <sub>24</sub> H <sub>36</sub> O <sub>2</sub>	356
16	32.16	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	1.07	C <sub>18</sub> H <sub>26</sub> O <sub>3</sub>	290
17	32.33	Methyl 9-eicosenoate	1.12	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324
18	32.84	Methyl-9,9,10,10-D4-octadecanoate	0.81	C <sub>19</sub> H <sub>34</sub> D <sub>4</sub> O <sub>2</sub>	302
19	33.99	à-N-Normethadol	0.41	C <sub>20</sub> H <sub>27</sub> NO	297
20	35.84	4H-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	0.43	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610
21	36.02	Sarreroside	0.36	C <sub>30</sub> H <sub>42</sub> O <sub>10</sub>	562
22	37.34	9,12,15-octadecatrienoic acid (Linolenic acid)	0.39	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>	496
-	-	<b>Total</b>	<b>100</b>	-	-

RT= relation time in minutes; M. F. = molecular formula; M. W. = molecular weight in g/mole.

**Table 2:** Effect of VCM, PPE, and VCM combined with PPE on the erythrogram.

Parameters	Control	VCM	PPE	VCM+PPE	<i>p-value</i>
<i>Erythrogram</i>					
RBCs (x10 <sup>6</sup> /μl)	6.19±0.13	5.00±0.22 <sup>a</sup>	6.12±0.17 <sup>b</sup>	5.39±0.15 <sup>ab</sup>	0.0001 <sup>***</sup>
Hb (g/dl)	14.45±0.21	12.41±0.38 <sup>a</sup>	14.84±0.16 <sup>b</sup>	13.75±0.36 <sup>bc</sup>	0.0001 <sup>***</sup>
Hct (%)	33.94±0.67	28.36±1.11 <sup>a</sup>	33.55±0.75 <sup>b</sup>	30.91±0.69 <sup>abc</sup>	0.0001 <sup>***</sup>
MCV (fl)	54.89±0.46	57.11±0.86 <sup>a</sup>	54.94±0.71 <sup>b</sup>	54.90±0.41 <sup>b</sup>	0.048 <sup>*</sup>
MCH (pg)	23.41±0.32	24.90±0.40	24.35±0.58	24.43±0.30	0.104
MCHC (g/dl)	42.66±0.41	43.93±0.55	44.36±0.91	44.53±0.24	0.123

**Table 3:** Effect of VCM, PPE, and VCM combined with PPE on the platelets.

Parameters	Control	VCM	PPE	VCM+PPE	<i>p-value</i>
<i>Platelets</i>					
Platelets (x10 <sup>3</sup> /μl)	852.13±53.47	805.88±58.03	769.75±39.26	861.38±56.07	0.580

**Table 4:** Effect of VCM, PPE, and VCM combined with PPE on WBCs, and the differential leukocyte counts.

Parameters	Control	VCM	PPE	VCM+PPE	<i>p-value</i>
<i>Leukogram</i>					
WBCs (x10 <sup>3</sup> /μl)	11.32±0.79	14.30±1.02	12.26±0.78	11.96±0.64	0.084
Neutrophils (%)	19.39±0.64	29.14±1.65 <sup>a</sup>	18.51±1.86 <sup>b</sup>	32.38±0.86 <sup>ac</sup>	0.0001 <sup>***</sup>
Lymphocytes (%)	73.66±1.44	63.56±2.67 <sup>a</sup>	74.16±1.76 <sup>b</sup>	59.94±1.72 <sup>ac</sup>	0.0001 <sup>***</sup>
Monocytes (%)	6.95±0.51	7.30±0.56	7.33±0.44	7.68±0.56	0.807

**Table 5:** Effect of VCM, PPE, and VCM combined with PPE on the neutrophil/lymphocyte ratio.

Parameters	Control	VCM	PPE	VCM+PPE	<i>p-value</i>
Neutrophil to Lymphocyte ratio (%)	0.26±0.01	0.46±0.03 <sup>a</sup>	0.25±0.0 <sup>b</sup>	0.54±0.02 <sup>abc</sup>	0.0001 <sup>***</sup>

- Number of animals (n) = 8 for all groups.
- Data tabled as mean±SE. *P*<0.05 was recognized as significant.
- The characters a, b, c, d, and e were used to represent the significant differences as follows:
  - <sup>a</sup> significant difference with control.
  - <sup>b</sup> significant difference with VCM.
  - <sup>c</sup> significant difference with PPE, respectively.
- \* *P* ≤ 0.05; \*\* *P* ≤ 0.01; \*\*\* *P* ≤ 0.001.

As for DIHA, the most frequently implicated drug categories are antibodies, nonsteroidal anti-inflammatory drugs

“NSAIDs”, and anticancer medicines [28, 29]. Drugs effects on RBCs range from subclinical hemolytic anemia to life-

threatening DIHA [30]. Consequently, the observed decrease in Hb levels is due to the reduction of RBCs. While with Htc % representing the RBCs volume percentage. As the quantity of RBCs falls, so does the percentage of Htc [31]. The high MCV in the VCM-treated rats indicates larger RBCs, likely a response to the reduced RBC as seen with gentamycin in rabbits [32]. Reduced RBCs result in less oxygen availability, and subsequent hypoxia. An increase has been observed in the MCV of RBCs in individuals exposed to intermittent hypoxia, which is evidence of augmented, young blood cells in the bloodstream or the adaptation of old ones to release damaged protein molecules in the vesiculation process [33].

Regarding the findings of the leukogram, there are observed significant increments in the WBCs and the neutrophils counts, besides the increase in the monocytes count, however, it was not statistically significant in the VCM-treated group. In other words, there was an increase in the neutrophil count at the expense of the lymphocyte count in this group, which is also known as the neutrophil-to-lymphocyte ratio (NLR). These results were consistent with Balkrishna *et al.* [19] who noted a significant rise in the lymphocyte count.

Leukocytes are composed of various cell populations, namely granulocytes (basophils, neutrophils, and eosinophils), monocytes, and T and B lymphocytes, each with specific immunologic roles. Neutrophils are the most common circulating cell population in the immune system and are a major component of the innate immune reaction. They are normally the first responders to acute inflammation and contribute to the resolution of inflammation [34, 35]. Whereas lymphocytes primarily mediate adaptive immunity and have an important contribution to the regulation of an appropriate inflammatory response [36, 37].

These significant elevations may be attributed to the VCM-induced inflammation cascade. It was stated by Kucukler *et al.* [38] and Al-Sroji *et al.* [21] that VCM induced free radical production and oxidative stress that trigger the nuclear factor kappa B (NF- $\kappa$ B) signaling pathway, which activates the inflammation cascade by activating inflammatory cytokines, such as tumor, interleukin 1 beta (IL-1 $\beta$ ), and Necrosis Factor-alpha (TNF- $\alpha$ ).

VCM-induced inflammation, possibly through oxidative stress triggering the NF- $\kappa$ B signaling pathway, increases inflammatory cytokines like IL-1 $\beta$  and TNF- $\alpha$ , contributing to kidney and liver cytotoxicity [21] [38]. Low lymphocyte counts indicate reduced cellular immunity, reflecting their migration to tissues for enhanced immune surveillance and sustained systemic inflammation [39, 40]. High NLR is a marker of systemic inflammation and stress [41].

The NLR, calculated as the ratio of neutrophils to lymphocytes, is a reliable marker of immune response to various triggers, reflecting systemic inflammation [42, 43]. High NLR is characterized by increased neutrophils and decreased lymphocytes, commonly seen in advancing inflammatory conditions [44, 45].

The current results indicate that PPE can attenuate the side effects of VCM, likely due to its bioactive components. Based on the GC-MS analysis, the PP aqueous extract was rich in (E)-9-octadecenoic acid methyl ester an anti-oxidant fatty acid [46], and methyl 9-cis, 11-trans-octadecadienoate, also known

as cis-9, trans-11 conjugated linoleic acid, a polyunsaturated fatty acid, and anti-inflammatory properties [47]. Also, it possesses anti-carcinogenic, anti-atherogenic, and positive immune modulatory properties [48]. PPE also contains other fatty acids like stearic acid methyl ester and hexadecanoic acid, both with antioxidant and anti-inflammatory properties [49, 50].

Besides, Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl is a flavonoid with strong antioxidant and antifungal activity [51], with potent anti-inflammatory demonstrated the utmost binding affinity versus inflammatory protein Intralukin-6 (IL-6) target [52]. Based on the information provided in the previous discussion, the potential underlying mechanisms by which PPE mitigates VCM-induced hematological toxicity are as follows:

### 1. Antioxidant Properties:

The GC-MS analysis revealed that PPE is rich in bioactive compounds such as (E)-9-Octadecenoic acid methyl ester, methyl 9-cis,11-trans-octadecadienoate, and 9-octadecenoic acid (E)-. These fatty acids and their derivatives are known to possess potent antioxidant activities. The antioxidant properties of PPE likely help to counteract the oxidative stress and damage induced by VCM, thereby protecting the hematological system. The presence of these antioxidants may neutralize reactive oxygen species (ROS), reducing oxidative stress and preventing the resultant cellular damage [53, 54].

### 2. Anti-inflammatory Effects:

The bioactive compounds in PPE also contribute to its anti-inflammatory properties. This is evident from the normalized neutrophil-to-lymphocyte ratios observed in the VCM + PPE group, indicating a reduction in inflammation. By mitigating the inflammatory response associated with VCM administration, PPE can help preserve the integrity and function of the haematological system. Inflammatory cytokines and markers, typically elevated during VCM-induced stress, were likely suppressed by the anti-inflammatory constituents of PPE [55, 56].

### 3. Maintenance of RBC Indices:

PPE co-administration was able to restore the RBC count, hemoglobin content, and hematocrit % that were reduced by VCM treatment. This suggests that PPE has a protective effect on RBC production, maturation, and/or survival, thereby preventing the haematological toxicity induced by VCM. The compounds in PPE may support erythropoiesis or enhance the survival of RBCs by protecting them from oxidative damage [57, 58].

### 4. Modulation of Leukocyte Profiles:

VCM administration altered the differential leukocyte counts, but PPE co-treatment was able to normalize these changes. This

indicates that PPE can help maintain the balance and function of different types of WBCs, which is crucial for the proper functioning of the hematological system. The modulation of leukocyte profiles suggests that PPE may influence leukocyte production, differentiation, or survival, contributing to an overall balanced immune response [4] [59]. Further mechanistic studies would be needed to elucidate the precise pathways and cellular mechanisms by which the bioactive compounds in PPE exert their protective effects against VCM-induced hematological toxicity. These studies could explore the specific signaling pathways involved in oxidative stress and inflammation, as well as the direct impact of PPE on bone marrow function and leukocyte dynamics. Understanding these mechanisms would provide deeper insights into the therapeutic potential of PPE and its application in preventing or mitigating drug-induced hematological disorders.

## 5. Conclusion

In conclusion, this study demonstrated that PPE possesses significant ameliorative effects against VCM-induced haematological toxicity in male Albino rats. The administration of VCM led to a noticeable decrease in RBC count, Hb content, and Hct %, alongside an increase in MCV, indicating oxidative stress and subsequent hemolysis as the underlying mechanisms. Additionally, VCM treatment resulted in elevated WBC and neutrophil counts, contributing to a heightened NLR, reflecting systemic inflammation.

The bioactive compounds identified in PPE, including (E)-9-octadecenoic acid methyl ester, methyl 9-cis,11-trans-octadecadienoate, and various fatty acids, likely confer its protective properties. These compounds exhibit strong antioxidant and anti-inflammatory activities, which may counteract the oxidative stress and inflammatory responses induced by VCM.

Our findings suggest that PPE can effectively mitigate VCM-induced haematological alterations, highlighting its potential as a natural therapeutic agent to combat drug-induced haematotoxicity.

## 6. CRediT author contribution statement

Conceptualization, H. Hassanine, H. M. Omar, M. K. Mahfouz, and R. A. Mahmoud, Methodology, H. Hassanine, H. M. Omar, M. F. El-Sayed, M. K. Mahfouz, and R. A. Mahmoud, Software, R. A. Mahmoud, and M. K. Mahfouz, Validation, H. Hassanine, H. M. Omar, M. F. El-Sayed, M. K. Mahfouz, Formal analysis, R. A. Mahmoud, Investigation, M. F. El-Sayed, M. K. Mahfouz, and R. A. Mahmoud, Resources, H. Hassanine, M. F. El-Sayed, M. K. Mahfouz, Data curation, R. A. Mahmoud, Writing-original draft preparation, R. A. Mahmoud, Writing- review and editing, H. Hassanine, H. M. Omar, M. F. El-Sayed, M. K. Mahfouz, Visualization, R. A. Mahmoud, Supervision, H. Hassanine, H. M. Omar, M. K. Mahfouz. All authors have read and agreed to the published version of the manuscript.

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