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Hematotoxicity induced by thioacetamide and the protective effect of date palm pollens (DPP) and Femara in male albino rats

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Abstract: Thioacetamide (TAA) is an organic compound that is used in rubber and pesticides production and it is considered as carcinogen. Femara ® (Letrazole) is a synthetic aromatase inhibitor (AIs) used in the treatment of breast cancer which work as competitive inhibitor of aromatase in peripheral tissues. Date palm pollen (DPP) is an important source of natural antioxidant which is largely related to the presence of flavonoids and vitamins. The current study aimed to investigate the interaction impact between DPP and Femara against the toxicity of TAA on the hematological parameters after four months in male albino rats. Seventy male albino rats were divided into seven groups. The first group act as a control group, the second group was treated orally with Femara (50 mg/kg body wt), the third group was treated (IP) with 100 mg/kg body wt. of TAA, the fourth group was treated orally with 100 mg/kg body wt. of DPP, the fifth group was treated with DPP before TAA, the sixth group was treated with DPP before TAA, whereas the seventh group was treated with Femara combined with DPP before TAA. The obtained results indicated that TAA induced a remarkable alteration, either increase or decrease in the hematological parameters relative to that of control. Moreover, DPP and Femara each alone or combined together normalized the hematological indices. So, it can be concluded that combination of Femara and/or DPP along with TAA have a beneficial impact in retarding the hematotoxicity of TAA in male rats.
Keywords: Thioacetamide, Femara, date palm pollen, hematological indices, albino rats.

1. Introduction

The World Health Organization (WHO) has estimated that over a quarter of the total global disease burden is linked to environmental factors, including exposure to toxic chemicals. Currently, tens of thousands of different chemicals are being utilized, with hundreds more being introduced annually [1]. Thioacetamide (TAA) (CH_3CSNH2) is a synthetic, colorless crystalline solid that is soluble in water and ethanol [2]. During the manufacturing of various synthetic compounds, including pharmaceuticals and pesticides, it is used as a sulfur donor. Additionally, its ability to release sulfides in aqueous solutions ensures its widespread use in analytical chemistry and sewer system cleaning [3]. Farmers are exposed to TAA in the pesticides through inhalation, ingestion or dermally, and distributed through the circulatory system to affect various organs. Hence, health hazards resulting from human exposure to these chemicals, especially from agricultural areas of developing countries have been a growing concern [4]. TAA undergoes a comprehensive metabolism, leading to the generation of sulfoxide and sulfone, which circulate through critical organs such the liver, kidney, and bone marrow [5]. Numerous studies have confirmed the validity of TAA hepatotoxicity and hematological toxicity [6].

Herbal medicine is one of the approaches to prevent, manage, and treat cancer that focuses on the naturally occurring chemical substances from plants for improving health conditions with minimal side effects in comparison with chemotherapy [7]. Date palm pollen (DPP), (*Phoenix dactylifera* L.) is the meal origin powder among the palm tree branches that is commonly consumed orally in the Middle East, the ancient Chinese and Egyptians for various therapeutic purposes. Also, it is considered as an effective natural and functional dietary food supplement [8, 9].

Phytochemical studies have shown that DPP contains a very wide range of biochemical and nutritional substances, such as essential and non-essential amino acids, trace elements, fatty acids as well as flavonoids [10]. It has been reported that DPP is a good source of natural antioxidants [11] and has several effects such as protecting erythrocytes from oxidative damage [12], anticoccidial, anti-apoptosis [13] and anti-breast-cancer [9]. Femara®, is the marketing name of Letrozole, manufactured by Novartis Pharmaceuticals Corp. Letrozole is a third-generation aromatase inhibitor (AI) used in the treatment of breast cancer. By inhibiting estrogen production, it reduces the development or causes the regression of hormone-responsive breast cancers in vivo [14]. Moreover, *in vitro* inhibit the proliferation of HCC cell lines [15]. Given these properties, the present study was designed to find out the role of Femara and date palm pollen supplementation in alleviating hematological toxicity induced by TAA in male albino rats.

2. Materials and methods

2.1. Chemicals and Tested drug and plant source

Femara® 2.5 mg (Letrazole tablets) for oral administration. It is chemically described as 4,4'-(1H-1,2,4-Triazol-1-ylmethylene) dibenzonitrile, and manufactured by Novartis Pharmaceuticals Corp., Cairo, Egypt. It is a white to yellowish crystalline powder, practically odorless.

Date palm pollen (DPP) grains were obtained from the Faculty of Agriculture farm, Sohag University, Egypt, dried and dissolved in distilled water using a sonicator. The DPP solution was administrated as an aqueous suspension via oral gavage.

Thioacetamide (TAA) ≥99.0% product number 163678, CAS number 62-55-5, MDL number MFCD00008070, formula CH_3CSNH_2, molecular weight75.13 was purchased from Sigma –Aldrich Company (St. Louis, MO, USA). TAA was prepared freshly by dissolving precious stones in sterile refined water and blending until all stones were broken up.

2.2. Animals

For the investigation, 70 adults male Wistar rats weighing 180–200 g was used. Animal House, Faculty of Science, Sohag University, Sohag, Egypt, is where the animals were purchased. The rats were kept in stainless steel cages beded on ssawdust in an air-conditioned room with a 12-hour light/12-hour dark cycle at a temperature of 25°C±2°C, and humidity about 15% throughout the experiment. The animals were given unlimited access to fresh water and nutrition.

2.3. Experimental design:

After a week of adaptation, the animals were arbitrarily allocated into seven groups, (n=10 animals/group), as follows: Group 1:acted as control group provided with water and food *ad libitum*. Group 2: The second group was oral administrated with Femara through a stomach tube at a dose of 50 mg/kg body wt. [16]. The third group was oral administrated of DPP through a stomach tube at a dose of 100 mg/kg body wt. [17]. Whereas the fourth group was treated with TAA intraperitoneal administration (IP) at a dose of 100 mg/kg body wt. [18]. The fifth group was treated with Femara through a stomach tube along with TAA (IP), while the sixth group was treated with DPP along with TAA(IP). The seventh group was treated with combined Femara and DPP along with TAA. All the treatments were administrated 3 times a week. The animals were observed daily in their cages for clinical signs. The experimental protocol was approved by the Ethical Committee of Sohag University (CSRE-2-24).

2.4. Collection of blood samples

After 4 months, 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). The following hematological parameters were measured; complete blood count (CBC), including red blood corpuscles (RBCs), hemoglobin (Hb), hematocrit (Hct %), Red Blood Cell Width deviation(RDW.SD),Red Blood Distribution-Width Coefficient of Variation (RDW.CV). Total white blood cell count (WWBCs) and differential leukocytes (monocyte, lymphocyte and neutrophil counts) and Lymphocyte neutrophil ratio (N/L), platelets count (PC) , mean platelet volume (MPV), platelet (PCT), platelet distribution width (PDW) and mean platelet volume to platelets count (MPV/PC) ratio using an automated hematological analyzer (Celltac, Japan) at Target Clinical lab, Akhmim, Sohag, Egypt.

2.5. Statistical analysis:

The data was first checked for normality and homogeneity. The Shapiro-Wilk W test was used to evaluate normality, while the homogeneity of variances test was used to check for homogeneity. To determine if there were any statistically significant differences between the experimental groups, a one-way analysis of variance (ANOVA) was performed. The statistical analysis was carried out using the SPSS 21.0 software program [19]. A Duncan's multiple comparisons post hoc test was used to compare the mean values of the various groups following the ANOVA. In the event that the p-value was less than 0.05 ($p < 0.05$), statistical significance was presumed. Mean values plus or minus the mean's standard deviation (Mean ± SD) are used to report the results.

3. Results and Discussion:

3.1. Hematological parameters:

The complete blood count of rats treated by Femara (50 mg/Kg body wt.) and DPP (100 mg/Kg B.W.) for four months causes a slight not significant difference in all RBCs indices compared to control group (**Table 1 and 2)**. The results of rats exposed to TAA (50 mg /Kg B.W.) for four months leads to a significant ($P < 0.0001$) decrease in most of RBC's indices including RBCs count, hemoglobin, HCT% and MCH levels compared to control, Femara and DPP-treated groups (**Table 1)**. In addition to remarkable decrease in MCHC compared to control group caused by TAA administration. Meanwhile MCV, RDW-SD and RDW-CV level in TAA -treated group is significantly ($P < 0.001$) higher than that in control, Femara and DPP groups (**Table 2)**.

In respect to the group which was treated with Femara plus TAA and DPP plus TAA groups, the count of RBCs and the levels of hemoglobin, HCT% , MCH and MCHC were significantly ($P < 0.0001$) lower than that of control, Femara and DPP-treated groups. Femara plus TAA treatment caused a significant ($P < 0.002$) increase in hemoglobin compared with TAA-treated group. While, Femara caused a significant ($P < 0.001$) increase in MCV and RDW-CV compared to control, Femara, DPP and TAA- treated groups. On other side DPP plus TAA-treated group caused significant ($P < 0.0002$) increase in MCV, RDW-SD, and RDW-CV compared to control, Femara and DPP-treated groups. Also, the aforementioned treatment caused a significant ($P < 0.0002$) increase in RDW-SD, and RDW-CV compared to TAA (**Table 2)**. (RDW.SD), Red Blood Cell Distribution – Width Coefficient of Variation (RDW.CV).

Finally, the combination of Femara and DPP in the treatment of TAA exposed rats resulted in significant decrease in RBCs, HB, and HCT levels compared to control, Femara and DPP groups (**Table 1)**. Moreover, MCHC showed a significant decrease compared with that in Femara and DPP

groups. In other hand MCV is significantly increase compared to control and Femara group. Also, the increase in RDW-SD and RDW-CV levels was high significantly different with control, Femara and DPP groups (**Table 2)**. Comparing to TAA group the combination of Femara and DPP treatment resulted in significant increase in RBCs count, HB, HCT and MCH level **(Table 1, 2).**

Leukocytes analysis showed a brief decrease in TWBC in femara treated group that attributed to significant decrease in neutrophil count compared to negative control group. This led to significant decrease in N/L ratio compared to control group too. However, DPP treated group showed significant increase in neutrophil count compared to control and femara treated group which led to the increase in TWBC and N/L ratio. Also, TWBC exhibited a remarkable significant increase in TAA treated group compared to control, Femara, and DPP treated group **(Table 3).** This increase on account of the elevation in lymphocyte and monocyte in TAA treated group compared to control, Femara, and DPP treated groups. Also, neutrophil count was significantly increased by TAA treatment compared to control and femara groups. Exposed rats to TAA treated with Femara and DPP displayed significant increase in WBCs, neutrophils and monocytes compared to control, Femara, and DPP treated groups and has no effect on WBCs indices and TAA- treated group. Femara plus TAA group showed significant (P 0.002) increase in monocyte with Femara treated group and a significant (P 0.005) increase in N/L ratio compared to control, Femara, and DPP- treated groups. DPPplus TAA led to significant (P 0.001) increase in monocyte compared to control, Femara, and DPP- treated groups and remarkable increase in N/L ratio compared to control, Femara and TAA -treated groups. Combination of Femara and DPP with TAA showed enhancement in neutrophils and N/L ratio compared to TAA- treated group and has no effect on lymphocyte and monocyte was documented. The treatment with TAA -induced nonsignificant increase in lymphocyte and neutrophil in respect to Femara and DPP in combination Platelets count of Femara and DPP -treated groups demonstrated a non-significant difference compared with control group except for PCT in Femara- treated group exhibited significance increase (P 0.002) ,compared with control group. TAA treatment led to significant decrease (P 0.001)in PT and MPV/PC ratio compared to control, Femara and DPP- treated group **(Table 4).** TAA - treated group exhibited also significant increase (P 0.001) in MPV, compared to control, Femara and DPP- treated group, and significant decrease (P 0.0001) in PCT compared to Femara and DPP -treated groups. Treatment with TAA and with Femara has no effect on platelets count, MPV, PCT, and MPV/PC ratio, compared to TAA- treated group. However, Femara plus TAA caused a significant increase (P 0.0001) in PDW, compared with control, Femara, DPP, TAA -treated groups. Whereas DPP combined with TAA led to enhancement in platelets count and PCT, compared to TAA -treated group, significant increase (P 0.0002) in PDW, compared to TAA-treated group and has no effect on mean MPV and MPV/PC ratio. Ultimately, Femara plus DPP together with TAA induced a brief enhancement in platelets, MPV, PCT, PDW and MPV/PC ratio.

4. Discussion

Blood act as a pathological reflector of the status of the exposed animals and humans to toxicants and other conditions. Clinical investigation of the presence of metabolites and other constituents in the body can be conducted through blood examination, which plays an essential role in physiology, nutrition, and pathology [20].

Table 1: The impact of Thioacetamide (TAA), Femara, date palm pollen (DPP), Femara plus TAA, date palm pollen plus TAA and Femara combined with date palm pollen plus TAA on **Red Blood Count** in Male Albino rats (*Rattus rattus*).

Groups	RBCs(10^6/ul)	HB(g/dl)	HCT (%)	M.C.H. (pg)
Control a	5.28 ±0.49	13.38 ±0.34	30.18 ±1.95	24.52 ±1.64
Femara b	5.33 ±0.56	13.46 ±0.57	28.08 ±1.99	24.45 ±2.03
DPP c	5.45 ±0.46	13.56 ±0.68	29.22 ±1.25	24.53 ±1.56
TAA d	3.74 ±0.62 a b c	9.61 ±1.04 a b c	22.70 ±1.43 a b c	21.48 ±1.32 a b c
Femara+TAA	3.95 ±0.71 a b c	10.66 ±0.61 a b c d	21.08 ±1.50 a b c	22.08 ±1.12 a b c
DPP+TAA	3.78 ±0.55 a b c	8.98 ±0.67 a b c	21.28 ±2.60 a b c	21.78 ±2.27 a b c
Femara+DPP+TAA	4.55 ±0.27 a b c d	10.68 ±0.95 a b c d	25.11 ±1.78 a b c d	23.62 ±1.36 d
P-Value	0.0001***	0.0001***	0.0001***	0.001**

Table 2: The impact of Thioacetamide (TAA), Femara, date palm pollen (DPP), Femara plus TAA, date palm pollen plus TAA and Femara combined with date palm pollen plus TAA on Red Blood indices in Male Albino rats (*Rattus rattus*).

Groups	M.C.H.C.(g/dl)	M.C.V.(Fl)	RDW-SD (fL)	RDW-CV (%)
Control a	43.87 = 2.45	53.50 ± 2.21	33.10 ±3.75	12.78 ±1.30
Femara b	45.16 = 2.39	52.41 ± 2.26	28.98 ±2.08	11.86 ±0.58
DPP c	47.38 = 2.55	54.55 ±2.51	30.21 ±2.79	12.03 ±0.62
TAA d	41.48 ± 3.65 c	58.49 ±2.71 a b c	37.73 ±3.92 a b c	13.46 ±1.16 a b c
Femara+TAA	35.30 ± 4.64 a b c	63.65 ±4.30 a b c d	45.28 ±2.63 a b c d	14.41 ±1.04 a b c
DPP+TAA	34.50 ±4.22 a b c d	60.56 ±3.64 a b c	47.40 ±5.02 a b c d	16.5 ±1.52 a b c d
Femara+DPP +TAA	38.08 ±3.11 a b c	58.03 ± 3.66 a b	38.05 ±2.90 a b c	14.73 ± 1.03 a b c
P-Value	0.001**	0.0001***	0.0001***	0.0001***

Table 3: The impact of Thioacetamide (TAA), Femara, date palm pollen (DPP), Femara plus TAA, date palm pollen plus TAA and Femara combined with date palm pollen plus TAA on **WBCs** in Male Albino rats (*Rattus rattus*).

Groups	TWBCs ($\times10^3$/UL)	Monocyte ($\times10^3$/UL)	Neutrophills ($\times10^3$/UL)	Lymphocyte ($\times10^3$/UL)	N/l ratio (%)
Control a	11.63 ±1.51	0.94 ± 0.16	2.69 ± 0.54	8.34 ± 1.15	0.32 ± 0.05
Femara b	10.64 ± 1.01	0.81 ± 0.11	1.49 ± 0.48 a	8.30 ± 0.79	0.18 ± 0.07 a
DPP c	12.63 ± 0.85 b	1.00 ± 0.19	٢.٥٨ ± ٠.٧٦ b	8.19 ± 1.17	0.43 ±0.09 a b
TAA d	18.28 ± ٠.٩٩ a b c	1.28 ± 0.29 a b c	5.07 ± 1.22 a b c	11.81 ±٠.٨٤ a b c	0.42 ± 0.06 a b c
Femara + TAA	18.98 ± 1.97 a b c	1.18 ± 0.12 b	5.19±1.08 a b c	12.41 ±1.03 a b c	0.٨١ ± 0.09 a b c
DPP + TAA	19.51 ± 1.86 a b c	1.30 ± 0.27 a b c	6.19 ± 1.48 a b c d	11.85 ± 1.24 a b c	0.51 ± 0.07 a b d
Femara+ DPP +TAA	16.36 ± 1.41 a b c d	1.27 ±0.23 a b c	3.44 ± 0.76 b d	11.60 ± 1.31 a b c	0.30 ± 0.07 b c d
P-Value	0.0001***	0.001**	0.0001***	0.0001***	0.0001***

Table 4: The impact of Thioacetamide (TAA), Femara, date palm pollen (DPP), Femara plus TAA, date palm pollen plus TAA and Femara combined with date palm pollen plus TAA on **Platelets indices** in Male Albino rats (*Rattus rattus*).

Groups	Platelets(10^3/ul)	MPV (fl)	PCT (%)	PDW (fl)	MPV/PC Ratio (%)
Control a	797.16 ± 66.15	6.63 ± 0.38	0.51 ± 0.09	11.11 ± 0.52	.0083
Femara b	856.66 ± 66.20	6.61 ± 0.44	0.60 ± 0.06 a	11.00 ± 0.92	.0083
DPP c	804.33 ± 50.97	6.70 ± 0.62	0.55 ± 0.04	11.56 ± 1.21	.0077
TAA d	657.00 ± 26.27 a b c	7.53 ± 0.59 a b c	0.44 ± 0.09 b c	10.83 ± 0.92	.0114 a b c
Femara+TAA	669.16 ± 71.09 a b c	7.50 ± 0.67 a b c	0.45 ± 0.04 b c	13.87 ± 1.46 a b c d	.0113 a b c
DPP+TAA	765.00 ± 13.82 b d	7.65 ± 0.80 a b c	0.59 ± 0.06 a d	12.45 ± 1.01 a b d	.010
Femara+ DPP+TAA	720.66 ± 57.50 a b c	7.06 ± 0.57	0.48 ± 0.07 b	11.95 ± 1.08	.0099
P-Value	0.0001***	0.002**	0.0001***	0.001**	0.0001***

Where Number of animals (n) = 6 for all groups.
Data tabled as mean ± SD. *P<0.05* was recognized as significant.
*$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

- Data is represented as mean ± SD. The P-values reported are for a one-way ANOVA test.
- The letters **a**, **b**, **c**, and **d** were used to denote the significant differences based on the Duncan test at the 0.05 significance level. as follows:
  a for control vs. other groups, **b** for Femara vs. DPP, TAA, Femara+ TAA, DPP +TAA, and Femara+DPP+TAA, **c** for DPP vs. TAA, Femara +TAA, DPP+TAA, and Femara+DPP+TAA, **d** for TAA vs. Femara+ TAA, DPP+TAA and Femara+DPP+TAA

Mice receiving TAA supplementation exhibited reduced activity levels and signs of increased stress. According to the results of this experiment, TAA significantly decreased RBC count, Hb content, Hct %, MCH, and MCHC and significantly increased MCV. This data in line with **Alamri [21]** who found that TAA injection three times/week for 4 weeks (200 mg/kg) in male rats decreases red blood cells, hemoglobin content, and hematocrit. The results obtained by **Ebhohon et al. [22]** showed that administration of TAA (300 mg/kg b.w. i.p.). decreased the values of Hb concentration, packed cell volume, and red blood cells. Moreover, **Al-Attar [6]** found that exposure to TAA (50 mg/kg b.w. i.p) daily for one month. caused decreases in the values of RBC, Hb and Hct.

The observed result can be ascribed to a number of potential mechanisms associated with TAA's toxicity towards the liver and overall metabolic function. When TAA induce liver injury in animal models results in hepatocellular necrosis and hypoxia following its biotransformation to acetamide and TAA-S-oxide **[23]**. TAA's toxicity to the liver is associated with the production of reactive oxygen species (ROS), the influx of inflammatory cells, the release of nitric oxide and activation of NF-κB**[24]**. In this context, RBCs are continuously exposed to both endogenous and exogenous sources of ROS like superoxide and hydrogen peroxide (H_2O_2) which plays a significant role in damaging the RBC membrane and impairing its deformability. **[25]**. **Travlos** et al.**[26]** suggested that erythrocyte damage is may be related to direct oxidative injury to the red cells by the TAA or to the pitting function of the spleen. It is necessary to consider the partially oxygenated hemoglobin (Hb) molecules formed within RBCs during microcirculation, as they transport oxygen to tissues, have an elevated affinity for the RBC membrane. This can increase the rate of Hb autoxidation, generating additional ROS that are not fully neutralized by the RBC's antioxidant system. This source of RBC oxidative stress is believed to be a key factor contributing to RBC aging and removal from circulation.**[25]**. Besides, RBCs have a limited capacity to restore damaged cellular components due to the loss of protein expression that occurs during erythropoietic maturation**[27]**. A further plausible explanation concerns that long-term high-dose use of TAA induces bone damage in experimental animals **[28]**. So, the haematopoietic system's susceptibility to damage makes the decrease in RBC value a result of TAA's effect **[6].** So that, reduction in hemoglobin concentration and packed cell volume may be due to an increased rate of hemolysis and /or reduction in the rate of erythropoiesis**[21]**.

In the contrary, TAA administration led to remarkable increase in MCV. This can be explained due to prolonged stress which may compromise red blood cell function by altering their shape and reducing their flexibility and deformability**[29]**. As well, red cell distribution width (RDW) indices in TAA treated group showed remarkable increase compared to control, Femara and DPP groups. RDW a classical parameter used in the differential diagnosis of anemia, has recently been recognized as a marker of chronic inflammation and high levels of oxidative stress (OS). Currently, it is used in the differential diagnosis of anemia. Increased RDW, has been associated with iron deficiency and nutritional deficiencies (folate or vitamin B12) and to a large number of disorders such as cardiovascular disease, venous thromboembolism, cancer, diabetes, , liver and kidney failure and chronic obstructive pulmonary disease **[30]. Sousa *et al.*, [30]** reported that RDW is a significantly increased in Fanconi anemia, which characterized by progressive bone marrow failure. This increase has a strong correlation with anemia and is linked to thrombocytopenia and neutropenia. **Salvagno et al. [31]** have proposed that the increase in RDW could be the consequence of stress erythropoiesis, which mobilizes more immature erythropoietic cells from bone marrow in response to OS-related dysregulation of RBC homeostasis and peripheral RBC degradation.

The tabulated elevation in all WBCs caused by TAA, identical with **Chen *et al.* [32]** observation about TAA single intravenous injection (280 mg/kg) induced increased blood WBCs. Less leukocyte margination into vessel walls, less extravasation from the vasculature into tissues, and an increase in leukocyte precursor cells in the bone marrow or their enhanced release from bone marrow storage pools can all be attributed to these findings **[33].** This rise in TWBC is mostly attributed to increased neutrophils.this increase lead to supsequent in N/L ratio effected neutrophils, immune cells that originate and mature in the bone marrow, are rapidly recruited to infection sites from the bloodstream, utilizing mechanisms like phagocytosis, ROS, and neutrophil extracellular traps **[34].** This recruitment of neutrophils is also observed in sterile inflammatory conditions, such as hepatic ischemia/reperfusion injury, where they mediate damage in the post-reperfusion phase **[35]**. Infiltration/migration of neutrophils in the liver may be intermediated by Kupffer cells via activating compliments and releasing chemokines **[36]**. **Kuramochi *et al.* [37]** claimed that TAA adminstration is characterized by the induction of coagulation necrosis in centrilobular hepatocytes, with subsequent macrophage infiltration.

The assessment of N/R as an emerging biomarker in various diseases is a rapidly growing field of biomedical research **[38]**. Elevation in N/L ratio induced by TAA adminstration compared to control group is indicator for ongoing cancer-related inflammation which induces tumorigenesis as a chronic pathological response in predisposed subjects. Tumor initiation switches on the so-called “cancer-elicited inflammation”, through a pro-inflammatory cytokine and chemokine storm, determine, in turn, the recruitment of immune cells, induction of angiogenesis and shifting to the promoting phase **[39]**. Stimulation of tumor-associated macrophages, and tumor-

associated neutrophils causes metastatic progression and potentiates systemic neutrophilic inflammation.

Thrombocytopenia induced by TAA administration compared to control, femara and DPP groups is similar to the hematological findings by **Alamri** [39] who demonstrated that TAA exposure resulted in decline in platelet count. Furthermore, Lin et al. [40] found that thrombocytopenia induction is linked to acute liver injury caused by TAA. In addition, TAA therapy raised MPV levels in comparison to the control, Femara, and DPP groups. According to **Elsha et al.** [41], individuals with liver cirrhosis have a larger MPV, which lowers thrombopoietin and lowers platelet counts. The MPV/PC ratio was significantly higher in the TAA-treated group than in the control group as a result of the decrease in the PL count and the rise in MPV. A reduced platelet count and higher platelet consumption may occur from endotoxin-induced platelet activation, tumor necrosis, or malignant cells, which might cause the elevated MPV/PC ratio [42].

Administration of Femara as positive control have no significant effect on RBCs indices although it causes neutropenia compared to control group. This result is in accordance with **Cohen *et al.*** [43] who stated that Femara showed no dose-related effects on hematologic or biochemical parameters. Neutropenia represented the most common treatment-related adverse event reported in the patient population receiving palbociclib plus letrozole [44]. On the other hand, the administration of Femara to TAA-induced rats led to an elevation in hemoglobin, MCV, RDW-SD and PDW levels, relative to the TAA-treated and control group. This combination also causes remarkable decrease in M.H.C.H. compared to control group. Femara likely caused this result by blocking estrogen, a key regulator of mature hematopoietic cells [45]. **Emmanuelle *et al.*** [46] reported that estrogen deficiency specifically E2 markedly changes the balance of cells constituting bone marrow, bone structure and bone resorption activity. So, the combination of the effect of TAA on bone marrow and effect of Femara can result in larger abnormal maturated cells with less hemoglobin and a wide variation in size.

Regarding DPP plus TAA treatment, DPP not fully counteract TAA's effects on RBCs; only increase in MCV, RDW-SD, RDW-CV levels is observed. Similarly, this treatment demonstrated continuing increase in TWBC compared to control, Femara and DPP treated group indicated in the significance increase in neutrophils compared to TAA treated group. So, a significance increases in N/L ratio observed in DPP treated group compared to control, Femara and TAA treated. This result is in accordance with **Abuoghaba *et al.*** [47] who found that the increased total WBCs, lymphocytes, neutrophils count for treated bucks may be due to the vital role of DPP in enhancing the immune functions against stress-induced tissue inflammation by TAA. Also, DPP plus TAA administration causes observed increment in platelets and PDW levels. Evidence suggests that DPP can reverse hematological and liver function changes induced by toxic substances in laboratory animals [48].

Phytochemical analysis of DPP revealed the presence of several important constituents. Amino acids, the primary building blocks of proteins, were identified. Along with minerals like zinc, selenium, iron, and copper, rutin, and flavonoids like quercetin and catechin, the plant also includes a number of vitamins, including B1, B2, B12, A, E, and C [49]. The last group, receiving both Femara and DPP for treatment of TAA-induced toxicity, showed significant improvements in several blood parameters compared to the group treated with TAA alone. Specifically, they had notable increases in RBC, Hb, Hct, and MCH. Additionally, this combined treatment also enhanced TWBC and neutrophil counts. As a result, the ratio of N/L ratio, significantly decreased compared to the group that only received TAA. Besides, platelets indices indicated a brief but not significant enhancement. These results can be accounted for combined effect of Femara as estrogen inhibitor and DPPs active components which indirectly reduce oxidative stress induced by TAA administration.

Further research is necessary to fully understand how Femara and the active components in DPP protect against TAA-induced blood toxicity. Specifically, these mechanistic studies should investigate the cellular pathways involved, including those related to oxidative stress and inflammation. The direct effects of Femara and DPP on bone marrow function and white blood cell activity also warrant examination.

5. Conclusion

In conclusion, this study showed that combining Femara and DPP significantly mitigated TAA-induced hematotoxicity in male albino rats. The antioxidant and anti-inflammatory properties of DPP's bioactive compounds and the estrogenic inhibitor of Femara counteract the oxidative stress and inflammation caused by TAA. So, the present results suggest that the Femara and DPP combination effectively ameliorates TAA-induced hematological changes, demonstrating its potential as a natural therapeutic approach for drug-induced blood toxicity.

CRediT authorship contribution statement:

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Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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