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# Gallic Acid Exhibits Splenic Protection and Enhances Haematopoietic Indices in Doxorubicin Challenge: A Supportive Therapeutic Role.

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## Abstract

Doxorubicin (DOX) is a chemotherapeutic agent that exhibits splenotoxicity and induce hematopoietic suppression in cancer patients. Gallic acid is an antioxidant and anti-tumor agent that protects non-cancer cells. Sixteen rats were randomly grouped into four (4) sub-groups of 4 rats. The control received 0.1 ml/kg/ i.p of normal saline, DOX alone group received 15 mg/kg, i.p of doxorubicin weekly, while the Low and high dose Gallic acid groups were given 15 mg/kg.i.p. of DOX and then treated with 60mg/kg and 120 mg/kg of Gallic acid orally for two weeks respectively. The blood samples were collected, for the analysis of hematologic parameters using the One-way ANOVA. The spleen was harvested and prepared for light microscopy and stained using Haematoxylin and Eosin. The study showed that Gallic acid exerts significant increase in the HB, PCV, MCHC, and WBC in both the Low and high dose groups and splenic-protective effect against the toxicity of Doxorubicin. Hence, Gallic acid prevented both splenic toxicity and haematopoietic suppression. As a result, this finding recommends its adoption in prophylaxis and as treatment option for Doxorubicin chemotherapy-induced splenotoxicity and haematopoietic suppression.

**Keywords:** doxorubicin, splenotoxicity, Gallic acid, chemotherapy, antioxidant, anti-tumor, hematopioesis

## Introduction

Doxorubicin is a chemotherapeutic agent[1] used in the management of various tumours such as breast, lung, and bladder cancers[2]. Doxorubicin acts on cancerous cells via intercalation into the Deoxyribonucleic acid (DNA), disruption of topoisomerase-II-mediated DNA repair, and the generation of free radicals which damage cellular membranes and proteins[3,4]. However, it has been singled out for its association with cardiac and splenic toxicities[1,5]. Consequential to doxorubicin chemotherapy, splenic toxicity accompanied by haematopoietic suppression is evident, this leads to a reduction in the amount of red bone marrow and an increase in yellow bone marrow[6].

The spleen functionally serves as a storage pool of immune cells and removal of aging blood cells in the recycling of blood constituents hence, regarded as an organ of the reticuloendothelial and haematologic systems[7,8]. Thus, the spleen plays vital roles in foetal erythropoiesis from the 10th to the 25th week of gestation[8], and post-partum, where the principal physiological function of the spleen includes purification, iron metabolism, inhibition of infectious agents, storage pool for red blood cells and platelets[7]. The erythrocyte and platelets filtration is carried out by the splenic cords in the red pulp; whereas much larger and older cells are collected in the splenic cord and destroyed by macrophages present in the reticulum and sinus endothelium of the spleen[7,9]. The spleen red pulp are the reservoir of the macrophages that are responsible for the opsonization of pathogens[10], it also stores iron in the form of ferritin[11].

Doxorubicin has been associated with a vicious cycle of splenic-cardiac events resulting in flawed immune metabolism and irremediable macrophage toxicity[1]. Doxorubicin rescinds macrophages present in the spleen, which it does by reducing $CD169^{+}$ cells and causing mass loss in an acute and chronic settings[1,12]. It further results in stenocardia cachexia as a result of its suppressive impact on the physiological levels of cytokines and chemokines[1]. In addition, Doxorubicin-

induced oxidative stress in chemotherapy through oxidative stress cascade of events that negatively affects the spleen. Anti-oxidant agents are known to alleviate oxidative stress and restore oxidative balance[13]. These anti-oxidants are found in many plants' nuts, seeds, and barks which are viewed as vital sources of Gallic acid, a naturally occurring secondary metabolite[14,15.]

Gallic acid and its derivative are reportedly present as one of the many phenolic constituents of some fruits such as gallnuts, oak bark, sumac, grapes, and tea leaves[16]. Gallic acid is also widely used as a raw material in the production of inks, cosmetic, and pharmaceutical products[17]. Previous studies disclosed that Gallic acid has numerous properties, these include anti-fungal, anti-viral, anti-oxidant, and anti-cancer properties[18,19]. Its biological effects on cells is attributed to the the molecular constituents known as alkyl esters of the Gallic acid[20].

The adverse effect of doxorubicin as an anti-cancer medication when engaged for chemotherapy of different kinds of cancer is cardiotoxicity, as well as splenic-toxicity[1,21] and requires replacement of blood constituents during therapy[21]. This fact remains a concern for patients under chemotherapy, also healthy non-cancerous cells are mostly affected due to their slow rate of mitotic activity compared to the cancerous cells. Reports from research studies indicate Gallic acid exhibits cytotoxicity against malignant cells whereas non-cancerous cells remain unharmed [22]. Gallic acid is also reportedly used as a remote astringent in cases where there is internal haemorrhage[23]. It has been found to have anti-fungal properties[24], anti-microbial properties[25], and anti-inflammatory properties[26]. Gallic acid is a strong antioxidant and helps to reduce oxidative stress whereas doxorubicin can otherwise damage the cells and lead to cell death. At present there is a paucity of available literature that demonstrate the potential of Gallic acid against Doxorubicin on the spleen of Wistar rats. In this regard the study investigated the potential of gallic acid as a likely supportive therapy in doxorubicin chemotherapy.

## Materials and Methods

### Drug and Chemicals

Gallic acid was purchased from Sigma, Aldrich USA. Doxorubicin was procured from a registered pharmacy store in Enugu, Nigeria. Analytical and Standard graded chemicals were obtained from registered chemical stores in Enugu State, Nigeria

### Animal Handling

Sixteen (16) albino Wistar rats weighing between 160g -190g of aged 6-8 weeks were purchased and housed at the animal house of the Enugu State University of College of Medicine, Nigeria. The rats were housed in a well-aerated laboratory cage with soft wood shavings as bedding. They were allowed two weeks to acclimatize, and fed with animal feed. The animals were maintained under laboratory conditions (temperature 36°C, with relative humidity of 60-70 percent, and a 12-hour light-dark cycle). After two weeks of acclimatization, the rats were weighed and randomized into groups. The protocol for the conduct of the study was reviewed and approved by the Faculty Research Ethic Committee (ESUCOM/FBMS/ETR/2022/015) of Esucom.

### Experimental Design

The rats were randomly grouped into four (4) subgroups of 4 rats each. The control received 0.1 ml/kg of normal saline intraperitoneally for two weeks. Doxorubicin alone group received 15 mg/kg of Doxorubicin weekly intraperitoneally for two weeks. Low dose gallic acid (GA) group received 15 mg/kg/ i.p. of Doxorubicin weekly and treated with Gallic acid at a dose of 60 mg/kg per oral daily for two weeks. The High Dose GA group received 15 mg/kg/ i.p. of Doxorubicin weekly and treated with daily doses of Gallic acid at 120 mg /kg for two weeks orally.

### Estimation of haematological parameters

A day after the last day of administration (day 1 post-treatment) blood was collected via ocular puncture through the retro-orbital vein of the rats with the aid of capillary tubes into EDTA and plain bottles. The blood sample collected from each group was used for the evaluation of haematological indices using the following parameters (white blood cell, haemoglobin, platelets). The blood sample estimation was carried out using the methods described by Kone *et al.*,[27]. The data were statistically analysed using the SPSS Package. The difference in the average mean values for the collected data was determined by employing One-way ANOVA. Data were presented in the table as ± mean standard deviation and considering *p* values at $P \leq 0.05$ and $P \leq 0.001$.

### Tissue Processing

The abdomen was excised and the spleen were harvested. The spleen was immediately fixed in 10% normal saline for one week. Dehydration was carried out through ascending grades of alcohols (50%, 70%, 90%, and absolute alcohol) for 45 minutes each. The tissues were cleared in three changes of xylene for

45mins each. The cleared tissues were impregnated in a hot oven in three changes of molten paraffin wax for 30 minutes each. The impregnated tissues were embedded using an embedding mould and allowed to solidify. The paraffin block was trimmed with a blunt microtome knife and stocked on the wooden block for microtomy. The tissues were serially sectioned to form ribbons 3µm thick using a rotary microtome machine. The tissue sections were picked from a water bath and mounted on a glass slide and dried on the hot plate. The staining was carried out using clean slides and staining jars. Paraffin sections were dewaxed through two changes of xylene each for 3 min. Sections were rehydrated through descending grades of alcohol, two changes for 1min each. The sections were ringed in running tap water for 5 min and stained with Haematoxylin for 15 min. It was differentiated in 1ml acid alcohol for 1min and blued in running tap water for 15 min. The sections were counterstained with eosin for 1min before being rinsed in water for 15 min, and thereafter passed through ascending grades of alcohol for a minute and then absolute alcohol for 1min. It was later transferred to xylene in two changes each for 2 min, mounted in DPX and covered with coverslips then dried in the oven.

## Results

### Haematological findings

**Table 1: Red cell parameters before and after treatment in the test and control groups**

| | Control | | Doxorubicin alone | | Low Dose GA | | High Dose GA | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| RBC ($x10^6$ /µl) | 6.68 ± 0.33 | 5.47± 0.33 | 6.31 ± 0.31 | 3.88 ±0.15$^{*\beta}$ | 6.89± 0.42 | 3.33±0.12$^{**\beta\beta}$ | 7.15± 0.31 | 4.35±0.41$^{**}$ |
| HB (g/dL) | 14.43 ± 0.69 | 25.3 ± 1.10** | 14.04 ± 0.55 | 11.73 ± 0.44$^{*\beta\beta}$ | 14.48± 0.68 | 15.95±0.366$^{*\beta\beta}$ | 15.18±0.73 | 19.60± 1.05 |
| PCV (%) | 42.75 ±1.89 | 54.00 ± 1.35** | 42.00 ± 1.78 | 35.75±1.25$^{**\beta\beta}$ | 43.00± 2.12 | 44.25±1.65$^{\beta\beta}$ | 45.00±2.38 | 51.25± 1.49 |
| MCV (µm$^3$) | 64.08 ± 0.99 | 3.83 ±0.52** | 66.63 ± 0.60 | 2.34±0.21** ββ | 62.58± 1.12 | 2.32 ± 0.4$^{1**}$ | 62.95± 1.59 | 2.66±0.19$^{**}$ |
| MCH (pg) | 21.60 ± 0.23 | 1.18 ± 0.06** | 22.88 ± 0.34 | 0.63± 0.07** ββ | 21.08± 0.55 | 0.81 ± 0.03** | 21.23±0.310 | 0.93±0.01** |
| MCHC (g/dL) | 33.78 ± 0.17 | 40.09 ± 1.52* | 34.33± 0.53 | 30.69± 1.82 β | 33.65± 0.28 | 34.3±1.378 β | 33.73± 0.26 | 34.87± 1.18 |

The values are expressed as mean ± SEM for pretreatment and post-treatment. *,**,β, ββ denote significant variation at $P< 0.05$ and $P< 0.01$ respectively.

**Table 2: White cell parameters and Platelet before and after treatment in the test and control groups**

| | Control | | Doxorubicin alone | | Low Dose GA | | High Dose GA | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| PLT (µL) | 291.75 ± 35.38 | 248.25± 6.56 | 237.25± 19.55 | 193.75± 8.74 ββ | 253.75± 23.28 | 213.00±1.78 ββ | 393.73±179.88 | 223.25± 2.69 |
| WBC (µL) | 5.86 ± 0.48 | 10.35 ± 1.07* | 5.14± 0.94 | 4.85±0.46 ββ | 6.65 ± 0.97 | 6.70± 0.178 ββ | 8.49 ± 2.88 | 7.10±0.18 |
| NEUT. (µL) | 35.00 ± 1.83 | 70.00 ± 1.96** | 35.75 ± 5.02 | 52.00±1.83ββ | 39.50± 3.77 | 57.75±1.93* | 25.50 ± 7.90 | 59.75±1.65* |
| LYMP. (µL) | 63.25 ± 1.97 | 33.25 ± 1.11** | 62.25± 4.85 | 40.25±1.44β | 59.25± 3.90 | 33.75±1.31* | 72.75± 7.95 | 33.75±2.17* |
| MONO.(µL) | 1.75 ± 0.48 | 0.00 ± 0.00* | 1.75 ± 0.25 | 0.50± 0.289 | 1.25 ± 0.25 | 0.00±0.00* | 1.75 ± 0.25 | 0.00±0.00** |
| EOS. (µl) | 0.00 ± 0.00 | 0.25 ± 0.25 | 0.00 ± 0.00 | 2.25± 0.25** | 0.00 ± 0.00 | 1.75± 0.48* | 0.00 ± 0.00 | 0.50± 0.50* |

Differential values of White blood cells and platelet are expressed as mean ± SEM for pretreatment and post-treatment. *,**,β, ββ denote significant variation at $P< 0.05$ and $P< 0.01$ respectively

**Histological Result**

The histology of the spleen of group 1 showed the parenchyma or splenic pulp. This splenic pulp has two components: aggregations of white pulp, which are small areas in the parenchyma, surrounded by the larger red pulp. The white pulp consists of aggregations of lymphatic nodules containing germinal centers. Within each lymphatic nodule is a central artery. The red pulp consists of blood-filled sinusoids and splenic cords (Fig 1 and 2 G1).

The histology of the spleen of groups 2-4 also showed similar structures as group 1. In group 2, the white pulp appeared to be denser in population with enlarged arteries, and more diffused within the red pulp, compared to group 1 (Fig I and II G2). In group 3, the white pulp appeared to be denser in population, and more aggregated, compared to group 1(Fig I and II G3). In group 4, the white pulp appeared to be denser in population, and more aggregated with enlarged central entry, compared to group 1(Fig 1 and 2 G4).

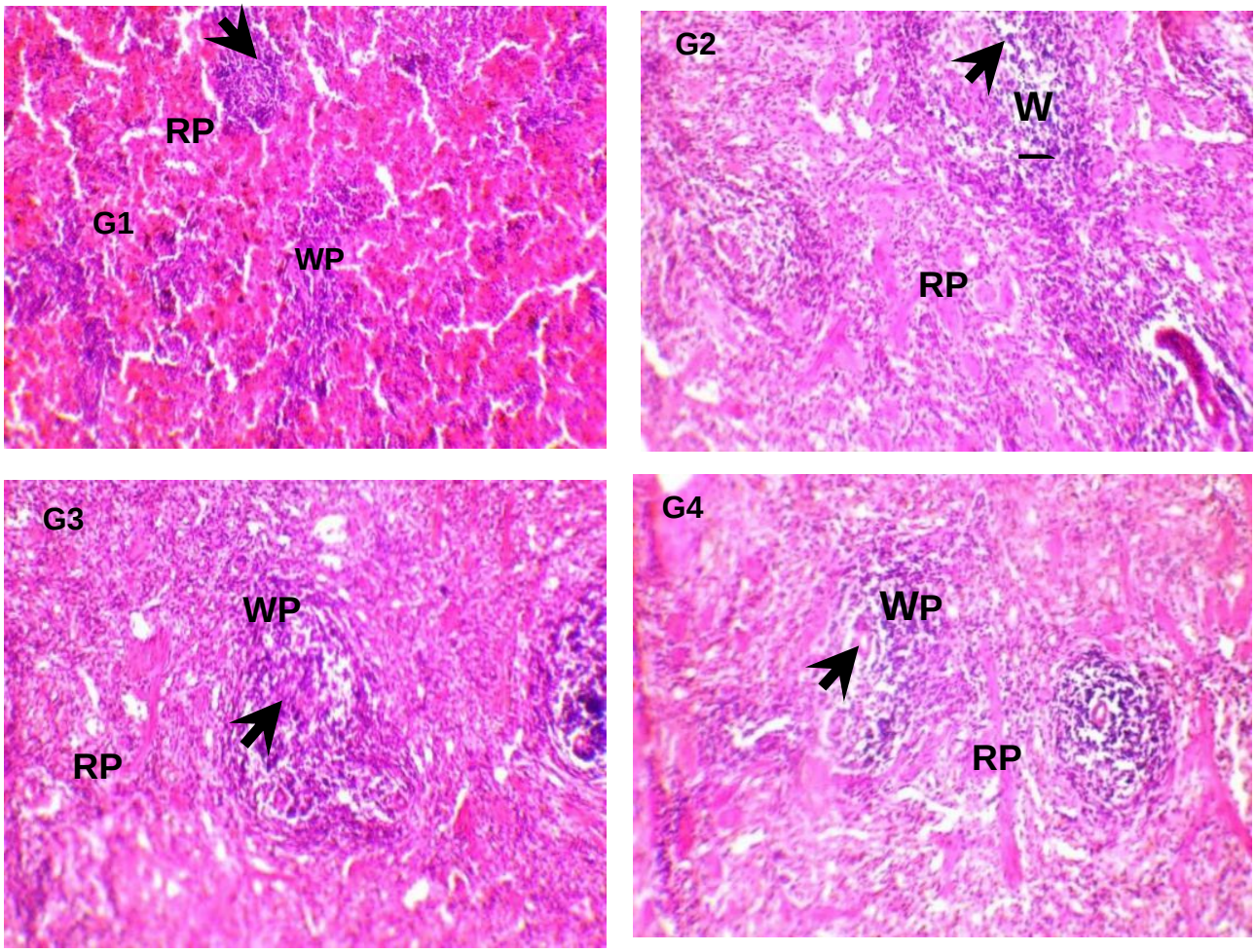


**Fig. 1:** Section of the spleen of G1: Control, G2: Doxorubicin alone, G3: DOX + Low Dose Gallic acid G4: DOX + High Dose Gallic acid (RP – Red pulp; WP – White pulp; Arrow – Central artery). H and E. MgX:100.

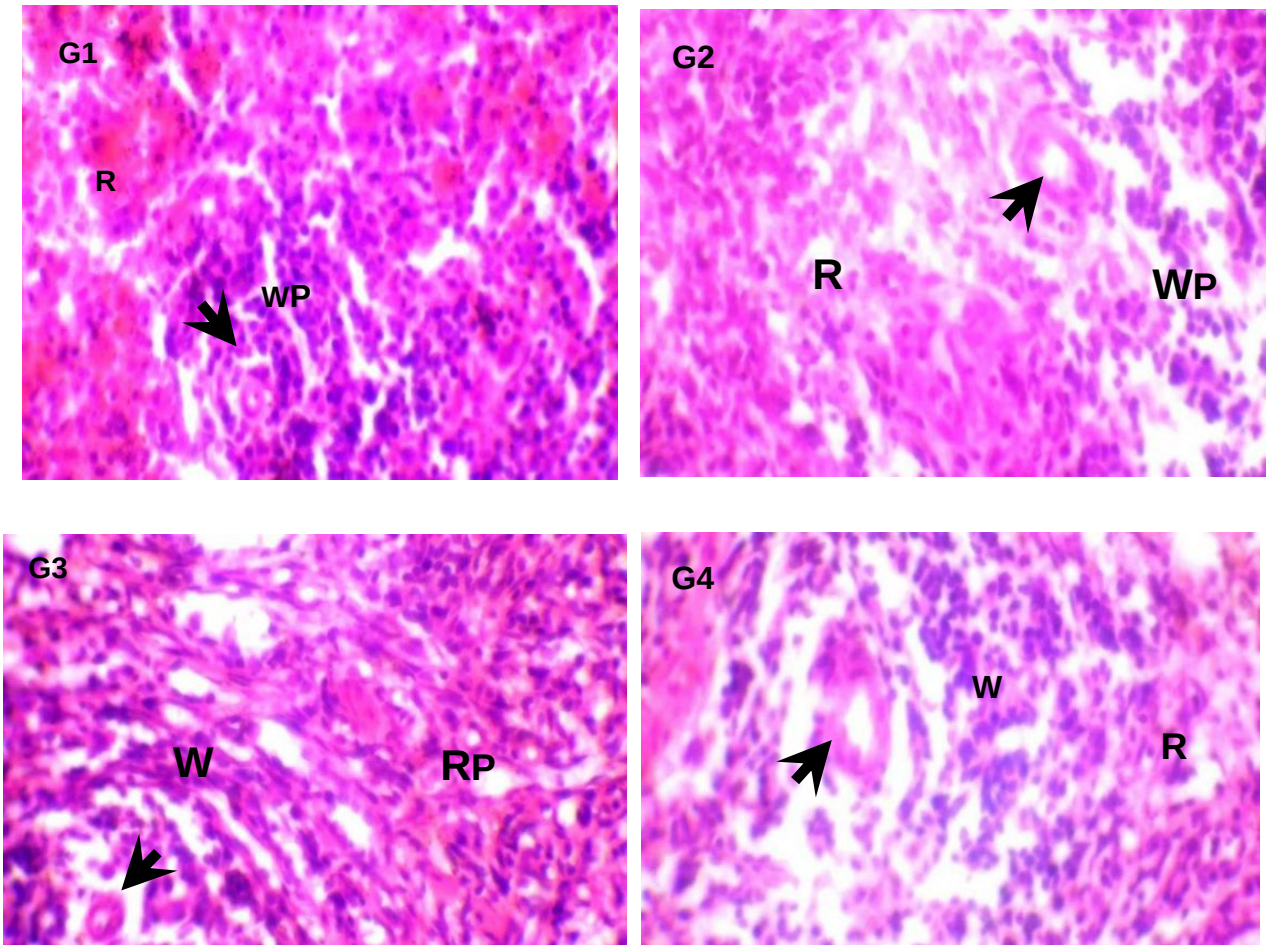


**Fig. 2:** Section of the spleen of G1: Control; G2: Doxorubicin alone; G3: DOX + Low Dose Gallic acid; G4: DOX + High Dose Gallic acid (RP – Red pulp; WP – White pulp; Arrow – Central artery). H and E. MgX:400.

**Discussion**

Anaemia is a haematological abnormality that is severe and recurrent in oncology patients[28]. Chemotherapy-induced anaemia (CIA) is an anaemic condition with numerous causative factors such as patient-based factors and treatment-associated factors which may include the chemotherapeutic agent, its administered dosage, and and the schedule of the successive therapy plan[29]. The complete blood count is a vital test to assess the side effects of chemotherapy.

In agreement with the splenic toxicity and haematopoietic suppression of DOX reported during its use as a chemotherapeutic agent[6], our findings showed that DOX reduced the levels of the haematological parameters, Red blood cell (RBC), Haemoglobin (HB), Mean Cell Haemoglobin Concentration (MCHC), Packed cell volume (PCV), Mean Cell Haemoglobin (MCH), White blood cell (WBC), Lymphocytes (LYMP), Monocytes Platelets (PLT). Except for Eosinophilia (EOS) and Neutrophils (NEUT). Eosinophilia is present in allergies, infections, and certain inflammatory conditions[30].

The estimation of white blood cell parameters for the DOX-treated group and its comparison with the pretreatment groups showed that the DOX had a significant impact on all the blood cell parameters investigated; however, this impact exempts monocytes where it had no significant impact. Likewise, previous studies had indicated that monocytes in the presence of DOX maintains the form of the pretreatment group concerning its metabolism amongst other features[31]. Investigating the effect of Gallic acid, it showed that Gallic acid altered the haematological parameters by showing a significant increase in HB, PCV, MCHC, EOS, and NEUT, when compared with the pretreatment group. However, it showed a significant decrease instead for RBC, MCV, MCH, PLT, LYMP, and MONO when compared with the pretreatment group. This finding is in agreement

with an earlier study carried out in chicks where Gallic acid altered the haematological parameters with a concurrent increase in PCV and HB counts[32].

Comparing the impact of DOX and Gallic acid on the haematological parameters, it is established that there is a significant difference in the impact of Gallic acid and DOX in the haematological parameters investigated. The findings showed a significant decrease in HB, PCV, MCHC, and WBC when compared with the pre-treatment group, unlike the Gallic acid group which showed a significant increase in the haematological parameters HB, PCV, MCHC, and WBC in both the low and high dose groups. This suggests that Gallic acid had a positive impact on the haematological parameters over DOX in chemotherapy. Similarly, DOX has been reported to cause alteration and reduction in haematological parameters[33]. The decrease in the blood parameter experienced with the DOX has been earlier attributed to its destructive effect on the organs of the body, lysing impact, myelosuppression, and hypoplasia[33,34]. The impact of Gallic acid on the haematological parameters is dose-dependent, this is evident in the comparison of the Gallic acid treated groups where it established that there is a significant difference in the impact of Gallic acid at the treatment dose on the haematological parameters over DOX for PCV at *P*-value < 0.05 but not for the high dose groups when compared, likewise, a significant impact for the high dose effect of Gallic acid over DOX for the HB and PCV at *P*-value < 0.01 were recorded both for the high dosed groups but not for the low dose groups. Hence, suggesting that Gallic acid has a dose-dependent impact on HB and PCV over DOX for chemotherapy.

The histological examination established an enlarged central artery for the DOX-treated group as well as the high dose group, this can however not be defined as aneurysmal since splenic artery is defined as aneurysmal when a focal dilation observed has a diameter greater than 50% as the extent of dilation was not determined. The findings also reported a similar result to Jadapalli *et al.*[1] which indicated that DOX induces splenic expansion of the red pulp on splenotoxicity. Likewise, this finding indicated that DOX causes the spleen to be more diffused within the region of the red pulp; however, this was not recorded for the groups where Gallic acid was administered. This suggests the protective potency of the Gallic acid on the spleen to DOX-induced toxicity and a concurrent impairment in the inflammation response program resulting from DOX immunosuppression[1,35].

## Conclusion

Gallic acid mitigates Doxorubicin splenic toxicity, preventing its hematopoietic and immunosuppressant effects. It significantly improved the haematological parameters associated with Chemotherapy-induced anaemia. Therefore, this study recommends the use of Gallic acid both as a prophylaxis and a treatment option for the management of Doxorubicin-induced splenotoxicity and haematopoietic suppression during chemotherapy.

## Conflict of Interest

The authors declare no conflict of interest

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## Authors Contribution

Conception and design FBE. supervised research FBE, wrote the draft OCJ and FBE, analysed and performed the histology OCE, and OCJ, AOE contributed to the discussion, reviewed, and edited the draft. FBE is the corresponding author. All authors read and approved the manuscript.

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