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EFFECTS OF AQUEOUS LEAF-EXTRACTS OF CHROMOLAENA ODORATA AND TRIDAX PROCUMBENS ON DOXORUBICIN-INDUCED HEMATOLOGIC TOXICITIES IN WISTAR RATS

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Abstract

This study investigated the influence of aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens* on haematological indices of doxorubicin treated rats. Doxorubicin (15 mg/kg body weight) was intra-peritoneally administered 48 h prior to sacrifice; while metformin (250 mg/kg), and the extracts (50, 75 and 100 mg/kg) were orally administered daily for 14 days. The red cells, white cells, lymphocytes and platelets counts, haematocrit, mean platelet volume, platelet distribution width, plateletcrit and platelet-larger cell ratio of Test control were significantly ($p < 0.05$) lower than those of Normal control, but not significantly lower than those of all the other groups. The mean cell haemoglobin and mean cell haemoglobin concentration of Test control were significantly ($p < 0.05$) higher than those of the other groups. The extracts had no harmful effect on the number of red cells, white cell and platelets indices, and prevented/ameliorated doxorubicin-induced haematological toxicity.

Introduction

The successful use of doxorubicin in chemotherapy has been limited, largely due to its diverse toxicities, including ocular (CARVALHO et al. 2009), cardiac (CARVALHO et al. 2009, SHOUKRY et al. 2017, AFSAR et al.

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2017, Zilinyi et al. 2018, Ahmed et al. 2019b), renal (Carvalho et al. 2009, Ahmed et al. 2019b, Bordbar et al. 2019), hepatic (Carvalho et al. 2009, Ahmed et al. 2019a, Alghorabi et al. 2019, Song et al. 2019), pulmonary (Jagetia and Lalrinpuii 2018), haematological (Sleijfer et al. 2018). Administration of doxorubicin has toxic effects on hematopoietic cells (Sleijfer et al. 2018), with the concomitant haematological toxicities such as anaemia, leukopenia, neutropenia and thrombocytopenia (Sleijfer et al. 2018). The haematological toxicity produced by doxorubicin is accompanied by reduced total erythrocytes count (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Khiavi et al. 2019, Islam et al. 2020), total white cells count (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer et al. 2018, Khiavi et al. 2019), platelets count (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer et al. 2018, Khiavi et al. 2019), lymphocytes count (Fayyaz et al. 2017, Fathy et al. 2018, Islam et al. 2020), granulocytes (neutrophils, eosinophils and basophils) count (Afsar et al. 2017, Fayyaz et al. 2017, Sleijfer et al. 2018, Khiavi et al. 2019), mid-sized cells (or monocytes) count (Fayyaz et al. 2017), mean corpuscular volume (Afsar et al. 2017), haematocrit (Afsar et al. 2017, Fathy et al. 2018, Islam et al. 2020), haemoglobin concentration (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer et al. 2018, Islam et al. 2020), mean cell haemoglobin (Afsar et al. 2017) and mean cell haemoglobin concentration (Afsar et al. 2017). Fathy et al. (2018) also reported increased neutrophils and monocytes counts; while Afsar et al. (2017) reported increased lymphocytes counts.

Doxorubicin suppresses the replicating precursor cells of the bone marrow resulting in reduced production of red blood cells (Khiavi et al. 2019) and leucocytes (Sleijfer et al. 2018), and can cause blood clotting disorders, anaemia and leukopenia (Khiavi et al. 2019). Therefore, its effect on blood parameters should be closely monitored (Khiavi et al. 2019). The management of doxorubicin-induced hematotoxicity is quite essential, hence the need for the investigation of herbal medications with potential preventive and ameliorative properties.

The leaf-extracts of *Chromolaena odorata* and *Tridax procumbens* are two of such preparation from plants, with the potential for the amelioration and prevention of doxorubicin-induced haematological toxicity. The leaves and their extracts have moderately high contents of iron, magnesium, flavonoids (e.g. quercetin, catechin and ellagic acid), saponins, tannins and other polyphenolic compounds (Igboh et al. 2009, Ikewuchi and Ikewuchi 2009b, Ikewuchi 2012a,b, Ikewuchi et al. 2009, 2012, 2013, 2014a,b, 2015), all of which are known modulators of haematological indi-

ces. The anti-anaemic property of flavonoids (e.g. quercetin) was reported by SEN et al. (2005). Increases in haematocrit, haemoglobin concentration and red cell count have been reported to result from magnesium supplementation (OTHMAN et al. 2016). Iron supplementation has been shown to raise haematocrit, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume, as well as red cells count and distribution width (DOGAR et al. 2013). Saponins and tannins have been reported to exhibit immunostimulatory activity and encourage lymphocytes proliferation (European Food Safety Authority 2009, DAVIDOVI et al. 2010, IKEWUCHI et al. 2011a, 2013, IKEWUCHI and IKEWUCHI 2013). Polyphenolic compounds were reported to be responsible for anti-thrombocytopenic effect of *Euphorbia hirta* (APOSTOL et al. 2012). Catechin and rutin were reported to be responsible for the anti-leukopenic and anti-thrombocytopenic activities of *Syzygium cumini* leaves (BANDIOLA and CORPUZ 2018). Ellagic acid was reported to cause increased platelet production by overexpression of cyclooxygenase pathway (ATTILIO et al. 2010).

The ability of the leaf-extracts of *C. odorata* and *T. procumbens* to positively modulate haematological indices, in salt-induced hypertensive and alloxan-induced diabetic rats were reported by IKEWUCHI and coauthors (IKEWUCHI 2012a, IKEWUCHI and IKEWUCHI 2013a, IKEWUCHI et al. 2014a). The leaf-extracts of *C. odorata* and *T. procumbens* have been reported to have anti-hypertensive (IKEWUCHI et al. 2010, 2011b, 2012), anti-dyslipidaemic (IKEWUCHI and IKEWUCHI 2009a, 2011, IKEWUCHI 2012a, IKEWUCHI et al. 2011c, 2014a,b), weight reducing (IKEWUCHI and IKEWUCHI 2009a, 2011, IKEWUCHI et al. 2010, 2011c), hepato-protective (IKEWUCHI 2012b, PALANISAMY et al. 2014), anti-diabetic (IKEWUCHI 2012a, ONKARAMURTHY et al. 2013), anticancer (VISHNU and SRINIVASA 2015, ADEDAPO et al. 2016) and antioxidant (PUTRI and FATMAWATI 2019, CUI et al. 2020) activities. In this study, the influence of aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens* on haematological indices was investigated in doxorubicin treated rats.

Materials and Methods

Procurement of Materials

Fresh samples of *Chromolaena odorata* and *Tridax procumbens* were collected from within the University of Port Harcourt, and were duly identified as previously reported (IKEWUCHI and IKEWUCHI, 2009b, 2011a,

2013, IKEWUCHI 2012a,b, IKEWUCHI et al. 2009, 2010, 2011a,b, 2012, 2013, 2014a,b, 2015). Forty five Wistar rats (weight 120–190 g) were obtained from the Animal House of Department of Pharmacology, University of Port Harcourt, Nigeria.

Preparation of Etracts

The leaves were rid of dirt. Then 6 kg of *C. odorata* and 5.5 kg of *T. procumbens* were macerated. The resultant extracts were dried in a water bath, and their residues (127 g and 116 g, respectively) were stored for use in the assay. The resultant leaf-extracts of *C. odorata* and *T. procumbens* (hereinafter referred to as COLE and TPLE, respectively), were weighed, reconstituted in distilled water and administered to the experimental animals, according to their individual weights and dosages of their groups.

Experimental Design

All experimental procedures in this study were performed in accordance with the ethical guidelines for investigations using laboratory animals, and complied with the guide for the care and use of laboratory animals (National Research Council 2011). The animals were weighed and sorted into nine groups of five animals each, with the average differences in weight ≤ 2.5 g (FAO 1991). They were housed in cages at the Department of Pharmacology, and allowed water and feed *ad libitum*. The animals were given standard rat chow product of Top Feeds Limited Nigeria

After 1 week acclimatization, the treatment commenced and lasted for 14 days. The animals were divided into nine groups of five rats each. Group 1 was normal control, Group 2 was test control, Group 3 was administered with Metformin or Diabetmin^{TM} (metformin HCl) (dissolved in distilled water) orally daily at 250 mg/kg body weight. This group is also referred to as reference drug group. The extracts were administered to groups 4–9 in the following order respectively; 50 mg/kg (COLE-50 mg), 50 mg/kg (TPLE-50 mg), 75 mg/kg to COLE-75 mg and 75 mg/kg (TPLE-75 mg), 100 mg/kg (COLE-100 mg) and 100 mg/kg (TPLE-100 mg). The test control group was administered with doxorubicin but was not given any of the extract while the normal control group was neither given doxorubicin nor treated with the extracts. Both received distilled water in place of the extract.

On day 12, doxorubicin was dissolved in normal saline and intra-peritoneally injected (15 mg/kg), into all the groups, except the normal control which was administered normal saline in place of doxorubicin solution. The doxorubicin dosage was adopted from SONG et al. (2019). The dosages

of administration of *C. odorata* extract was adopted and modified from IKEWUCHI et al. (2012, 2014a,b); that of *T. procumbens* extract was from IKEWUCHI et al. (2011b,c); while that of metformin was from ZILINYI et al. (2018).

Collection of Blood Samples and Determination of Haematological Indices

On day 14, the animals were sacrificed under chloroform anaesthesia and blood was collected into EDTA bottles for the haematological assay. Haematological indices were determined using Medonic M^{16} Haematological Analyser (Nelson Biomedical Limited, UK).

Statistical Analysis of Data

Statistical calculations were carried out with the Excel 2010 (Data Analysis Add-in) software. All data are expressed as mean ± standard error of the mean (SEM), and were analysed using one-way analysis of variance. Significant difference of the means was determined using least significant difference test; $p < 0.05$ was considered statistically significant.

Results

The effect the leaf-extracts of *C. odorata* and *T. procumbens* on platelet indices of doxorubicin treated rats is presented in Table 1.

Table 1
Effect of the leaf extracts of *Chromolaena odorata* and *Tridax procumbens* on platelet indices

Treatments	Platelets count [$\cdot 10^9$/L]	Mean platelet volume [fL]	Platelet distribution width [fL]	Plateletcrit [%]	Platelet-larger cell ratio [%]
Normal control	853.00±280.92^a	8.40±1.48^a	13.10±2.16^a	0.78±0.31^a	22.16±9.69^a
Test control	399.40±44.70^b	6.14±0.18^b	9.48±0.23^b	0.24±0.03^b	4.72±0.62^b
Metformin	472.50±24.92^{b,c}	6.18±0.12^b	9.45±0.16^b	0.29±0.01^b	6.95±0.81^b
COLE-50 mg	524.00±55.93^{a,b,c}	6.02±0.18^b	9.28±0.25^b	0.31±0.03^b	5.54±1.02^b
COLE-75 mg	402.60±119.70^b	4.64±1.18^b	8.92±0.25^b	0.30±0.04^b	3.69±0.67^b
COLE-100 mg	482.60±79.56^{b,c}	6.16±0.17^b	9.42±0.20^b	0.29±0.05^b	6.14±0.97^b
TPLE-50 mg	475.00±47.26^{b,c}	6.46±0.35^b	10.44±0.60^b	0.31±0.03^b	8.76±2.67^b
TPLE-75 mg	739.60±96.70^c	6.40±0.14^b	9.96±0.29^b	0.47±0.05^{a,b}	7.98±1.24^b
TPLE-100 mg	629.20±69.44^{a,b,c}	6.28±0.08^b	9.54±0.11^b	0.39±0.05^b	5.64±0.41^b

Values are mean ± SEM, $n = 5$ animals, per group. Values in the same column with different superscript letters differ significantly at $p < 0.05$

Table 2

Effects of aqueous leaf extracts of *Chromolaena odorata* and *Tridax procumbens* on red cell indices of doxorubicin treated rats

Treatments	Red cells count [$\cdot 10^{12}$/L]	Mean corpuscular volume [fL]	Red cell distribution width		Haematocrit [%]	Haemoglobin concentration [g/dL]	Mean cell haemoglobin [pg]	Mean cell haemoglobin concentration [g/dL]
			absolute value [fL]	per cent [%]				
Normal control	8.19±0.39^{a}	57.52±1.35^{a}	35.80±1.09^{a}	16.94±0.48^{a,b}	47.00±1.95^{a}	16.72±0.87^{a}	19.08±0.28^{a,c}	33.24±0.61^{a}
Test control	5.63±1.60^{b}	54.12±1.53^{a,b}	34.74±4.17^{a}	19.24±3.40^{a,b}	36.95±3.57^{b,c}	13.38±3.89^{a,b}	21.68±1.47^{b}	40.98±4.11^{b}
Metformin	7.61±0.24^{a,b}	57.40±2.03^{a,b}	36.03±1.14^{a}	16.98±0.44^{a,b}	43.53±1.17^{a,b}	14.58±0.40^{a,b}	19.20±0.45^{a,c}	33.53±0.44^{a}
COLE-50 mg	7.41±0.47^{a,b}	55.12±1.35^{a,b}	35.06±1.16^{a}	17.76±0.49^{a,b}	40.68±2.20^{a,b}	14.34±0.91^{a,b}	18.84±0.34^{a,c}	34.26±0.57^{a,c}
COLE-75 mg	6.65±0.35^{a,b}	56.06±1.15^{a,b}	35.14±1.55^{a}	17.60±0.67^{a,b}	37.24±2.06^{b,c}	12.74±0.52^{a,b}	19.14±0.40^{a,c}	34.36±0.59^{a,c}
COLE-100 mg	6.35±0.57^{a,b}	56.58±0.85^{a,b}	34.58±0.70^{a}	16.18±0.60^{a}	36.00±3.37^{b,c}	12.28±1.09^{b}	19.38±0.19^{a,c}	34.28±0.36^{a,c}
TPLE-50 mg	6.06±0.58^{b}	53.38±1.70^{b}	34.36±1.46^{a}	18.70±1.76^{a,b}	32.56±3.71^{c}	11.88±0.80^{b}	19.86±0.81^{a,b}	37.46±2.45^{a,b}
TPLE-75 mg	6.59±0.67^{a,b}	54.26±0.45^{a,b}	37.62±1.34^{a}	20.54±1.48^{b}	35.70±3.67^{b,c}	13.64±0.94^{a,b}	20.94±1.46^{b,c}	39.00±2.47^{b,c}
TPLE-100 mg	6.96±0.49^{a,b}	53.70±1.76^{a,b}	33.70±1.70^{a}	17.20±0.57^{a,b}	37.66±1.81^{b,c}	12.60±0.77^{a,b}	18.18±0.25^{a}	33.96±0.81^{a,c}

Values are mean ± SEM, $n = 5$ animals, per group. Values in the same column with different superscript letters differ significantly at $p < 0.05$

Table 3

Effect of aqueous leaf extracts of *Chromolaena odorata* and *Tridax procumbens* on white cell indices of doxorubicin treated rats

Treatments	Total white cells count [$\cdot 10^9$/L]	Lymphocytes count		Granulocytes count		Mid-sized cells count	
		absolute value [$\cdot 10^9$/L]	per cent [%]	absolute value [$\cdot 10^9$/L]	per cent [%]	absolute value [$\cdot$ 10^9/L]	per cent [%]
Normal control	21.13±5.14^{a}	14.85±2.94^{a}	74.55±5.30^{a}	3.55±2.08^{a}	13.13±4.70^{a}	2.73±0.77^{a}	12.33±0.82^{a}
Test control	5.46±0.91^{b,c}	3.12±0.49^{b}	62.66±4.24^{a}	0.54±0.14^{a}	10.80±1.10^{a}	1.56±0.45^{a,b}	26.54±3.48^{a,b}
Metformin	3.78±0.72^{b}	2.43±1.01^{b}	53.03±16.10^{a}	0.23±0.11^{a}	10.50±6.58^{a}	1.13±0.45^{a,b}	36.48±17.01^{b}
COLE-50 mg	6.68±0.69^{b,c}	4.78±0.89^{b,c}	71.34±9.50^{a}	0.90±0.68^{a}	15.14±10.94^{a}	1.00±0.14^{a,b}	13.52±1.75^{a}
COLE-75 mg	4.70±1.18^{b}	3.82±1.09^{b,c}	76.64±7.17^{a}	0.28±0.10^{a}	12.48±6.41^{a}	0.60±0.27^{b}	10.64±3.52^{a}
COLE-100 mg	5.34±0.68^{b,c}	3.74±0.73^{b,c}	68.68±7.98^{a}	0.52±0.21^{a}	10.50±3.91^{a}	1.16±0.28^{a,b}	20.82±4.36^{a,b}
TPLE-50 mg	11.72±4.79^{a,b}	7.88±2.88^{a,b}	70.44±3.48^{a}	2.10±1.31^{a}	13.84±3.73^{a}	1.74±0.62^{a,b}	15.68±1.51^{a}
TPLE-75 mg	16.56±10.00^{a,c}	10.66±6.00^{a,c}	65.38±6.29^{a}	3.32±2.62^{a}	16.20±3.81^{a}	2.58±1.50^{a}	18.42±3.13^{a,b}
TPLE-100 mg	4.76±0.61^{b,c}	3.88±0.82^{b,c}	77.22±9.43^{a}	0.24±0.13^{a}	8.74±4.78^{a}	0.64±0.15^{b}	14.04±4.83^{a}

Values are mean ± SEM, $n = 5$. Values in the same column with different superscript letters differ significantly at $p < 0.05$

The platelets count of test control was significantly ($p < 0.05$) lower than those normal control and TPLE-75 mg; but not significantly lower than those of all the others. The mean platelet volume, platelet distribution width, plateletcrit and platelet-larger cell ratio of test control were significantly ($p < 0.05$) lower than those of normal control; but not significantly lower than those of all the other groups.

As shown in Table 2 the red cells count and haematocrit of test control were significantly ($p < 0.05$) lower than those of normal control, but not significantly lower than those of all the other groups. The mean cell haemoglobin and mean cell haemoglobin concentration of test control were significantly ($p < 0.05$) higher than those of all the other groups, except TPLE-50 mg and TPLE-75 mg. The haemoglobin concentration, mean corpuscular volume and red cell distribution width of test control were not significantly lower than those of all the other groups.

Table 3 shows the effect of aqueous leaf-extracts of *C. odorata* and *T. procumbens* on white cell indices of doxorubicin treated rats. The total white cells count of test control was significantly ($p < 0.05$) lower than that of normal control; but not significantly lower than those of all the other groups. The lymphocytes count of test control was significantly ($p < 0.05$) lower than those of normal control and TPLE-75 mg; but not significantly lower than those of all the other groups. The granulocytes and mid-sized cells counts of test control were not significantly lower than those of all the other groups.

Discussion

The present result is in agreement with earlier reports of doxorubicin-induced reduction in haematological parameters such as: total erythrocytes counts (Afsar et al. 2017, Fathy et al. 2018, Khiavi et al. 2019, Islam et al. 2020), total white blood cells count (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer et al. 2018, Khiavi et al. 2019), platelets count (Afsar et al. 2017, Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer et al. 2018, Khiavi et al. 2019), lymphocytes count (Fayyaz et al. 2017, Fathy et al. 2018, Islam et al. 2020), granulocytes (neutrophils, eosinophils, and basophils) count (Afsar et al. 2017, Fayyaz et al. 2017, Sleijfer et al. 2018, Khiavi et al. 2019), and mid-sized cells (or monocytes) count (Fayyaz et al. 2017). Others include mean corpuscular volume (Afsar et al. 2017), haematocrit (Afsar et al. 2017, Fathy et al. 2018, Islam et al. 2020), haemoglobin concentration (Fayyaz et al. 2017, Fathy et al. 2018, Luthfi et al. 2018, Sleijfer

et al. 2018, ISLAM et al. 2020), mean cell haemoglobin (AFSAR et al. 2017) and mean cell haemoglobin concentration (AFSAR et al. 2017). It however, negates the reports of doxorubicin-induced increases in lymphocytes (AFSAR et al. 2017), neutrophils and monocytes (FATHY et al. 2018).

The haemopoietic system of the test rats was beneficially affected by the extracts. The extracts mildly improved their red cell indices. Similar positive modulation of red cell indices or anti-anaemic effects of the extracts were reported by IKEWUCHI and colleagues in salt-induced hypertensive and alloxan-induced diabetic rats (IKEWUCHI 2012a, IKEWUCHI et al. 2014a). The effect of the extracts may be due to their enhancement of erythropoiesis or inhibition of doxorubicin-induced destruction of red cells, or prevention of doxorubicin-induced myelosuppresion, inhibition of hematopoietic tissues and/or defective iron metabolism (AFSAR et al. 2017, SLEIJFER et al. 2018).

The observed elevated red cells count produced by the extracts, though not dose dependent, is an affirmation of the fact that the elevated haemoglobin concentration is the product of elevated red cell mass. The capacity of the extracts to increase red cell indices in the treated animals may be attributable to the presence of magnesium, iron, and flavonoids (e.g. quercetin), hitherto reported in the leaves and their extracts (IGBOH et al. 2009, IKEWUCHI and IKEWUCHI 2009b, IKEWUCHI et al. 2009, 2012, 2013, 2015).

The extracts may have increased the white cells' count by encouraging lymphocytes proliferation (or lymphopoiesis), granulocytopoiesis and monocytopoiesis, and preventing doxorubicin-induced myelosuppresion (SLEIJFER et al. 2018). This anti-leucocytopenic (anti-lymphocytopenic, anti-granulocytopenic and anti-monocytopenic) effect of the extracts may be due to the presence of saponins and tannins, both of which were hitherto reported in the leaves and their extracts by IKEWUCHI and colleagues (IGBOH et al. 2009, IKEWUCHI, 2012a, IKEWUCHI et al. 2009, 2013, 2014a, 2015). Similar anti-leucocytopenic effect of the extracts on salt-induced hypertensive and alloxan-induced diabetic rats were previously reported by IKEWUCHI and colleagues (IKEWUCHI 2012a, IKEWUCHI and IKEWUCHI 2013, IKEWUCHI et al. 2014a). This mild/moderate increase in total white blood cells counts produced by the extracts is beneficial, because, in addition to enhancing immunological, antimicrobial and inflammatory responses (BENSON and CALIGIURI 2018, CARTY et al. 2018, DORSHKIND and RAWLINGS 2018, KHANNA-GUPTA and BERLINER 2018), they could also provide defence against the onset of acute coronary syndrome (MORENO et al. 1994, LIBBY 2001, AYALOGU et al. 2011, IKEWUCHI et al. 2011a, 2013b, IFEANACHO et al. 2020).

The extracts prevented doxorubicin-induced thrombocytopenia. They may have achieved this by enhancing platelets production and/or preventing doxorubicin toxicity on the platelets (ZUNJAR et al. 2016, AFSAR et al. 2017). Similar anti-thrombocytopenic effect by the extracts on salt-induced hypertensive and alloxan-induced diabetic rats were previously reported by IKEWUCHI and coauthors (IKEWUCHI 2012a, IKEWUCHI et al. 2014a). This anti-thrombocytopenic activity of the extracts may be due to the presence of polyphenolics (ATTILIO et al. 2010, APOSTOL et al. 2012, BANDIOLA and CORPUZ 2018). This mild/moderate increase in platelets counts evoked by the extracts indicates enhanced clotting and lowered bleeding (KAUSHANSKY 2009, IKEWUCHI et al. 2011a, 2013, CANTOR 2018, IFEANACHO et al. 2020).

In conclusion, the increase in haematocrit, red blood cells, total white blood cells, platelets and lymphocytes counts though not dose dependent, may signify the positive effects of the extracts on the haemopoietic system of experimental rats. This highlights the potential of the leaves in the management of doxorubicin-induced anaemia and immune-suppression, as well as for the improvement of the haematological abnormalities associated with doxorubicin-induced haematological toxicity.

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