European Journal of Medicinal Plants

30(2): 1-12, 2019; Article no.EJMP.52698
ISSN: 2231-0894, NLM ID: 101583475

Functional Iron Indices and Highly Sensitive CRP Post Single Dose Irradiation with Cobalt^{60} and the Ameliorating Effects of Single and Combined Doses of Aqueous Extracts of *Parquetina nigrescens, Camellia sinensis* and *Telfairia occidentalis* in Guinea Pigs

L. O. Olatunbosun[1*], E. A. G. Khalil[2], A. O. Shittu[1], F. D. Olalere[1], S. A. Lawal[3], S. A. Biliaminu[4], K. A. T. Ogunwale[5], E. O. Sanni[1], S. A. Atunwa[6] and M. A. Oyenike[7]

[1]Department of Haematology and Blood Transfusion, University of Ilorin Teaching Hospital, Ilorin, Nigeria.
[2]Department of Clinical Pathology and Immunology, Institute of Endemic Diseases, University of Khartoum, Sudan.
[3]Department of Haematology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.
[4]Department of Chemical Pathology, University of Ilorin and University Teaching Hospital, Ilorin, Nigeria.
[5]Department of Chemical Pathology, University Teaching Hospital, Ilorin, Nigeria.
[6]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara, Nigeria.
[7]Department of Medical Laboratory Science (Histopathology and Morbid Anatomy), LAUTECH, Osogbo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors LOO and EAGK were involved in conceptualizing the research work and wrote the protocol. Authors LOO, EAGK, AOS and SAA wrote the protocol and the draft of the manuscript as well as carrying out the research work. Authors LOO, FDO, SAL, KATO and SAB were involved in the laboratory analysis, study design, data collation as well as analysis. Authors SAA, LOO and MAO were involved in animal care, extracts administration and toxicological assessment. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2019/v30i230173
Editor(s):
(1) Dr. D. Sivaraman, Sathyabama Institute of Science and Technology, Centre for Laboratory Animal Technology and Research, Chennai, India.
(2) Prof. Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) L. Hariprasath, Karpagam Academy of Higher Education, India.
(2) Maria Elena Calderón-Segura, National Autonomous University of Mexico (Universidad Nacional Autónoma de México), Mexico.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52698

**Corresponding author: E-mail: deluy008@gmail.com;*

Original Research Article

Received 16 September 2019
Accepted 26 November 2019
Published 05 December 2019

ABSTRACT

Background: The relationship between iron, hypoxia, inflammation, and erythropoietin in cellular homeostasis is well documented. Patients on radiotherapy are known with active immune/inflammatory disorders often accompanied with reduced iron uptake or unavailability of circulatory iron and hence, must be adequately evaluated. The present study hypothesized "aqueous extracts of *Camellia sinensis*, *Telfairia occidentalis* and *Parquetina nigrescens* have chemical properties of ameliorating and restoring to normal, functional iron deficiency sequel to Cobalt 60 irradiation effect".

Materials and Methods: Fifty-Five young male guinea-pigs approximately 450 gram in weight were recruited and thirty were randomly assigned to 3 groups (A, B and C) for the study. Groups A and B were further divided into 4 (A1-4 and B1-4) with 3 animals (n=3) per group. Three guinea-pigs were also assigned to group C. Groups A and B belonged to Pre and post-irradiation groups while groups C served as control. Each animal was given 400r (4.0 Gy) whole-body gamma-irradiation under general anaesthesia, using a Co^{60} therapy unit as a source. Groups A1, A2, A3 and A4 had 1,400 mg/kg *C. sinensis*, 4000 mg/kg *P. nigrescens*, 3,500 mg/kg *T. occidentalis* and Combined dose (1,400 mg/kg *C. sinensis* + 400 mg/kg *P. nigrescens* + 3,500 mg/kg *T. occidentalis*) respectively twice daily 72 hours prior to irradiation and continued throughout the 14 days of the study. Groups B1, B2, B3 and B4 had similar treatment but commenced 24 hours after exposure to radiation and likewise continued throughout the 14 days of the study. Group C were not given any treatment but also had irradiation.

Results: Total Iron Binding Capacity, Ferritin, Serum Transferrin receptor and Iron were all increased significantly for all the extracts pre and post irradiation. However, C-reactive protein decreased significantly.

Conclusion: Aqueous extracts of *Camellia sinensis, Parquetina nigrescens* and *Telfairia occidentalis* leaves have good ameliorating effect on irradiation-induced injuries.

Keywords: Camellia sinensis; Telfairia occidentalis, Parquetina nigrescens; functional iron indices; Cobalt^{60}.

1. INTRODUCTION

Apart from acute and transient bone marrow suppression which typically results from exposure to a moderate dose of total body irradiation (TBI), Inflammation is also a major complication of irradiation exposure [1]. Indirect support for pro-oxidant and pro-inflammatory effects of radiation was revealed by the fact that non-steroidal anti-inflammatory drugs and antioxidants can alleviate some of that latent damage, at least in vivo, as well as reduce inflammation-induced mutations [2,3]. Pro-inflammatory cytokines are important components of immediate early gene programs and as such are rapidly activated in the tissues after irradiation. The inflammatory response that ensues is maintained by the production of reactive oxygen species, cytokines, chemokines and growth factors along with inflammatory infiltrates [4,5]. These are responsible for most of the side effects of irradiation or radiotherapy. Both acute and chronic inflammations are recognized causes of fatigue secondary to anaemia, called anaemia of inflammation (AI) [1].

There are multiple players in the pathophysiology of AI: The excessive production of inflammatory mediators diverts iron to the mononuclear phagocyte system (MPS) rendering it relatively unavailable for erythroid progenitors [6]. Hepcidin anti-microbial peptide (HAMP), one of such inflammatory mediators is the hormonal negative-feedback regulator of serum iron, as it limits iron-fluxes to the circulation. It is release from the liver due to inflammation caused by irradiation. Hepcidin blocks iron absorption and inhibits its recycling from senescent red blood cells. Other inflammatory mediators released upon liver injury are IL 1 and 6R. IL-6 and other pro-inflammatory cytokines result in a downregulation of Transferrin expression in the

liver, thus reducing the serum's capacity to transport iron [7]. Erythropoietin (EPO) production in the kidney is also inhibited by inflammatory mediators such as tumor necrosis factor (TNF) and IL-1 [8,9]. Aside reduced production of EPO, there is downregulation of the EPO receptor on erythroid cells by interferon (IFN)-γ [10], another inflammatory mediator. Tumour Necrosis Factor (TNF), IL-1, IFN-γ, and other reactive intermediates inhibits the proliferation and differentiation of erythroid progenitors [11,12,13].

The combined effect of Hepcidin and these inflammatory mediators results into functional iron deficiency, whereby there is reduced availability of iron but with normal or increased iron reserve, while EPO deficiency further inhibits incorporation of iron into erythroid progenitor, Burst Forming Unit Erythroid (BFUe). With low Transferrin saturation, there is non-regenerative normocytic or microcytic anaemia.

Camellia sinensis is well consumed as a health-derived beverage from time immemorial and contains abundant phytochemicals, chiefly polyphenols which are regarded as strong antioxidants [14,15]. It is also well regarded as a medicine because of the reports by several studies as anti-mutagenic [16], anti-diabetic, anti-bacterial, anti-inflammatory, and lipid-cholesterol lowering properties [17,18]. The leave extract of *Telfairia occidentalis* (Cucurbitaceae) is used locally in the treatment of malaria and anaemia [19,20]. It exhibits anti-inflammatory [21] erythropoietic [22], anticholesterolemic [23] and antidiabetic activities [24,25] and as anticonvulsant in Nigeria [20]. *Parquetina nigrescens* (*Periplocaceae*) is a shrub common in West Africa and used for the treatment of gonorrhea and menstrual disorders [26]. The whole plant is used to stupefy fish in Ghana and Liberia, while the leaves and latex are used for the treatment of rickets, diarrhoea, skin lesions and tropical skin diseases [26]. The leaves of the plant have been used for the treatment of wounds, boils, and carbuncles in Africa [27]. Studies on *P. nigrescens* have shown promising anti-sickling effect in humans [28] and animals [29,30,31]. Also, anti-typhoid activity of ethanolic leaf extract of *P. nigrescens* was reported in mice [32,33].

Several recommendations have been presented regarding how to cope with radiation effects among which are drinking of at least 8 ounce glasses of water per day for diarrhea and dehydration, taken of tarts fruits or fruit-flavored sourballs in case of change of taste, eating small frequent meals to reduce nausea and vomiting and for patients with loss of appetite, rinsing of mouth with water before meals for patients with dry mouth and so on. Measures to ameliorate the hypoferremic condition promoted by inflammation will be of immense benefit to patients after radiotherapy or radiation injury also. It was in the light of this that we decided to assess functional iron indices post irradiation and evaluate the potential benefit of the aqueous extracts of *Camellia sinensis, Parquetina nigrescens, and Telfairia occidentalis* in single and combined doses since they have been reported in our previous study to have synergistic effects on bone marrow haemopoietic multipotent stem cells differentiation and proliferation post irradiation [34]. We also assessed the level of C - reactive protein (CRP) as an indication of on-going inflammatory process.

2. MATERIALS AND METHODS

2.1 Study Design

This was an experimental and interventional study.

2.1.1 Collection and Identification of the plant material

Fresh leaves of *Parquetina nigrescens* and *Telfairia occidentalis* were collected from University of Ilorin, Nigeria Plant Garden while a refined product of *Camellia sinensis* (purity and authentication certified by the regulatory body in Nigeria- National Agency for Food and Drug Administration and Control {NAFDAC}) was purchased from pharmaceutical premises in Ilorin, Nigeria. The plants were identified and authenticated by a taxonomist at the Department of Plant Biology, University of Ilorin, Nigeria. *Parquetina nigrescens* was given Serial Number 876 and Ledger Number 67 while Telfairia occidentalis was given Serial Number 959 and Ledger Number 150. Thereafter, collected samples were deposited in the herbarium of the institution for future references.

2.1.2 Processing of the plants' extracts

Four hundred grams and 350 g of the powdered leaves of *Telfairia occidentalis* and *Parquetina nigrescens* respectively were each soaked in distilled water in a closable container. The finished product (fine granules) of *Camellia sinensis* was also weighed and soaked in

distilled water. These were shaken for about 5 minutes and left to extract by means of maceration (shaking the mixture intermittently) at 28°C for 72 hours. The mixtures were filtered into a porcelain crucible using a fine mesh. The supernatant was concentrated below 40°C using rotary evaporator and then freeze-dried. The extract was stored at 4°C in freeze-dried form i.e the extract was freeze dried (Mitsubishi GOT 1000®) and then stored as such at 4°C for subsequent use in the experiment.

2.1.3 Preliminary phytochemical screening

The phytochemical constituent of the aqueous extracts were determined using standard procedures as described previously [35,36]. The extracts were tested for the presence of saponins, tannins, alkaloids, anthraquinones, cardiac glycosides, flavonoids and terpenoids.

2.1.4 Animal care

All Animals were kept in the animal house of the Toxicology Unit, Department of Pharmacology, Faculty of Pharmacy, University of Ilorin, Nigeria. The animals were kept in plastic cages (34 × 47 × 18 cm^3) in an air conditioned environment with one Guinea-pig in each cage. Animals maintained at room temperature of (25 ± 2)°C of relative humidity (60% ± 10%) under 12 hour night and light cycle. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for two weeks prior to experimental protocol to minimize if any of non-specific stress.

2.1.5 Animal grouping for the study

Fifty-Five young male guinea-pigs of mean weight 450 g were obtained from the animal house, Ladoke Akintola University of Technology (LAUTech) College of Medicine Osogbo, Osun-State, Nigeria were recruited and thirty were randomly assigned to 3 groups (A, B and C) for the study. Groups A and B were further divided into 4 (A1-4 and B1-4) with 3 animals (n=3) per group. Three guinea-pigs were also assigned to group C. Groups A and B belonged to Pre and Post-irradiation groups while group C served as control.

2.1.6 Administration of extracts

Groups A1, A2, A3 and A4 had 1,400 mg/kg *C. sinensis*, 4000 mg/kg *P. nigrescens*, 3,500 mg/kg *T. occidentalis* and Combined dose (1,400 mg/kg *C. sinensis* + 4000 mg/kg *P. nigrescens* + 3,500 mg/kg *T. occidentalis*) respectively twice daily 72 hours prior to irradiation and continued throughout the 14 days of the study. Groups B1, B2, B3 and B4 had similar treatment but commenced 24 hours after exposure to irradiation and likewise continued throughout the 14 days of the study. Group C were not given any treatment but also had irradiation plus saline.

2.1.7 Animal grouping and administration of extracts

Type of Animal Used = Guinea pig, Number of Animal Selected per Group = 3, Total number of used for the irradiation study = 30, Average Weight of guinea pig used = 450 g, Method of Administration = Oral.

2.1.8 Method and dose of irradiation

Irradiation was done at University College Hospital Ibadan, Oyo State, Nigeria by the method described by Harris [37]. After general anesthesia using intra-muscular ketamine 5 mg/kg body weight plus 1 mg Atropine, each guinea-pig was placed in a cotton-gauze bag and positioned lying on the side. Each animal was given 400r (4.0 Gy) whole-body gamma-irradiation under general anaesthesia, using a Co^{60} therapy unit as a source. The radiation technique is Source Skin Distance (SSD) at the depth of 4cm and dose rate of 3 Gy/1.53 minute.

2.2 Sample Collection

2 milliliters of venous blood was collected aseptically from the Lateral Saphenous vein from each animal on days 0 (day of irradiation), 3, 9 and 14 using the method described by Malene, et al. [38] according to protocols approved by the Danish Animal Experimentation Inspectorate under the Ministry of Food, Agriculture and Fisheries. The samples were dispensed into bottles containing EDTA and analyzed immediately.

2.2.1 After-care of the irradiated animals

To minimize the two major complications enumerated by Harris [37] i.e. the danger of internal haemorrhage from minor trauma and the risk of infection, resulting from the effects of irradiation on haemopoietic tissues, each irradiated animal were kept in a separate cage and excessive handling avoided until it was due for sacrifice. Each animal was adequately fed and given adequate supply of water.

Table 1. Description of doses given to different groups

Group (n = 3)	Time of Exposure to radiation	Saline	*C. sinensis*	*P. nigrescens*	*T. occidentalis*	Combined doses (in divided doses as twice daily)
1	Pre-irradiation (Group C)	10 ml/kg	-	-	-	-
2	Pre-irradiation (Group A (A1))		1,400mg/kg	-	-	-
3	Pre-irradiation (Group A (A2))		-	3,500 mg/kg	-	-
4	Pre-irradiation (Group A (A3))		-	-	400 mg/kg	-
5	Pre-irradiation (Group A (A4))	-	-	-	-	Dose/body weight
6	Post-irradiation (Group C)	10 ml/kg		-	-	-
7	Post-irradiation (Group B (B1))	-	1,400 mg/kg	-	-	-
8	Post-irradiation (Group B (B2))	-	-	3,500 mg/kg	-	-
9	Post-irradiation (Group B (B3))	-	-	-	400 mg/kg	-
10	Post-irradiation (Group B (B4))	-	-	-	-	Dose/body weight

2.3 Functional Iron Assessment

2.3.1 Serum iron & total iron binding capacity

Serum iron assay and Total iron binding capacity were determined together by colorimetric method using the commercially prepared Pointe Scientific, Inc iron/TIBC reagent, 2016. Serum Ferritin Assay was assessed using commercial kits supplied by Alpco:

2.3.2 Unsaturated Iron-Binding Capacity (UIBC)

Iron level + UIBC = TIBC (ug/dl)
SI Unit Conversion µg/dl × 0.179 = µmol/l.

2.3.3 Soluble Transferrin receptor assay

Soluble Transferrin Receptor Assay (sTfR) was assessed using commercially prepared reagents kit, Abnova, 2016.

2.3.4 C-Reactive protein

The quantitative detection of human C-reactive protein (CRP) in serum or plasma samples was determined using the human CRP ELISA kit, Affymetrix, 2016.

2.4 Data Analysis

Results were analyzed using SPSS version 2.0. Mean differences between tests and control and between pre and post irradiation values were compared using t-test with statistical significance considered at $p<0.05$.

3. RESULTS

3.1 Pre-irradiation Groups

For *C. sinensis*, the mean difference between controls and treated groups was significant from days 3 to 14 for TIBC (p-values 0.03, 0.00, 0.00), Ferritin (p-values 0.00, 0.00, 0.00) and STFr (p-values 0.00, 0.00, 0.00) while it only became significant on day 14 for serum iron (p-values 0.00), Table 2.

For *P. nigrescens*, the mean difference between controls and treated groups was significant from days 3 to 14 for all the indices, p-values 0.003, 0.00 and 0.00 for TIBC, 0.00, 0.00 and 0.00 for Ferritin, 0.01, 0.03 and 0.00 for STFr, 0.01, 0.00 and 0.01 for Iron and 0.03, 0.02 and 0.00 for CRP, Table 3.

For *T. occidentalis*, the mean difference between controls and treated groups was significant from days 3 to 14 for Ferritin (p-values 0.00, 0.00, 0.00), STFr (p-values 0.01, 0.00, 0.00), Iron (p-values 0.01, 0.00, 0.00) and CRP (p-values 0.00, 0.02, 0.00) while it became significant from day 9 to 14 for TIBC (p-values 0.00, 0.01), Table 4.

Table 2. Mean Difference (MD) between tests and controls of the effects of *Camellia sinensis* leaf extract on functional iron parameters pre-cobalt 60 irradiation

Day interval		TIBC	FERITIN	STFr	IRON	CRP
Day 0	MD	-20.03	-2.50	1.03	31.53	-0.33
	p-value	0.57	0.04	0.68	0.22	0.93
Day 3	MD	48.80	5.30	8.13	9.93	-24.33
	p-value	0.03	0.00	0.00	0.08	0.05
Day 9	MD	209.70	26.03	15.30	52.80	-27.67
	p-value	0.00	0.00	0.00	0.09	0.09
Day 14	MD	96.23	18.00	10.30	103.70	-26.67
	p-value	0.00	0.00	0.00	0.00	0.01

Table 3. Mean Difference (MD) between tests and controls of the effects of *Parquetina nigrescens* leaf extract on functional iron parameters pre-cobalt 60 irradiation

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	-12.67	-2.77	-0.47	23.07	2.33
	p-value	0.68	0.02	0.87	0.48	0.57
Day 3	MD	73.63	8.07	7.10	61.53	-30.33
	p-value	0.03	0.00	0.01	0.01	0.03
Day 9	MD	174.83	15.03	7.60	108.37	-43.67
	p-value	0.00	0.00	0.03	0.00	0.02
Day 14	MD	135.50	20.00	20.37	124.83	-30.33
	p-value	0.00	0.00	0.00	0.01	0.00

Table 4. Mean Difference (MD) between tests and controls of the effects of *Telfairia occidentalis* leaf extract on functional iron parameters pre-cobalt 60 irradiation

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	-13.43	-0.50	-0.70	-26.37	-3.33
	p-value	0.75	0.41	0.83	0.36	0.50
Day 3	MD	53.43	7.77	6.67	108.27	-64.67
	p-value	0.13	0.00	0.01	0.01	0.00
Day 9	MD	166.30	13.83	13.27	149.77	-46.33
	p-value	0.00	0.00	0.00	0.00	0.02
Day 14	MD	122.97	37.00	17.93	128.53	-33.67
	p-value	0.00	0.00	0.00	0.00	0.00

Table 5. Mean Difference (MD) between tests and controls of the effects of combined extracts of *Camellia sinensis, Parquetina nigrescens* and *Telfairia occidentalis* leave extracts on functional iron parameters pre-cobalt 60 irradiation

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	-30.47	-1.20	-0.57	1.93	0.00
	p-value	0.43	0.61	0.85	0.94	1.00
Day 3	MD	40.33	6.00	5.43	28.70	-29.33
	p-value	0.05	0.00	0.01	0.07	0.03
Day 9	MD	185.60	15.63	11.03	98.97	-42.67
	p-value	0.00	0.00	0.00	0.01	0.02
Day 14	MD	96.03	29.17	29.17	88.20	-27.00
	p-value	0.00	0.00	0.00	0.00	0.00

Table 6. Mean Difference (MD) between tests and controls of the effects of *Camellia sinensis* leaf extract on functional iron parameters post-cobalt 60 irradiation

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	3.17	-0.17	5.13	30.83	5.57
	p-value	0.92	0.88	0.07	0.22	0.12
Day 3	MD	71.73	3.93	4.80	36.23	-57.00
	p-value	0.00	0.00	0.01	0.02	0.01
Day 9	MD	70.00	21.07	14.83	122.20	-33.33
	p-value	0.00	0.00	0.00	0.00	0.01
Day 14	MD	83.97	18.77	15.87	101.73	-72.33
	p-value	0.00	0.00	0.00	0.00	0.00

Table 7. Mean Difference (MD) between tests and controls of the effects of *Parquetina nigrescens* leaf extract on functional iron parameters post-cobalt 60 irradiation exposure

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	-22.07	0.53	1.30	19.73	1.00
	p-value	0.52	0.68	0.58	0.48	0.75
Day 3	MD	118.30	7.73	6.60	46.97	-36.00
	p-value	0.00	0.00	0.00	0.01	0.04
Day 9	MD	145.80	20.60	21.13	182.20	-29.00
	p-value	0.00	0.00	0.00	0.00	0.00
DAY 14	MD	225.93	33.43	30.57	163.73	-54.00
	p-value	0.00	0.00	0.00	0.00	0.00

With combined extracts, the mean difference between controls and treated groups was significant for all the indices except iron. For TIBC, p-values were 0.05, 0.00 and 0.00, for Ferritin, p-values were 0.00, 0.00 and 0.00, for STFr, p-values were 0.01, 0.00 and 0.00, for CRP, p-values were 0.03, 0.02 and 0.00 while for iron it was 0.01 and 0.00, Table 5.

Table 8. Mean Difference (MD) between tests and controls of the effects of *Telfairia occidentalis* leaf extract on functional iron parameters post-cobalt 60 irradiation exposure

Day interval		TIBC	FERITIN	STFr	IronFe	CRP
Day 0	MD	-14.80	0.10	1.97	25.30	0.67
	p-value	0.63	0.96	0.42	0.40	0.87
Day 3	MD	72.57	7.40	7.10	60.47	-77.67
	p-value	0.04	0.00	0.01	0.01	0.00
Day 9	MD	153.77	14.63	12.33	134.23	-35.67
	p-value	0.00	0.00	0.00	0.00	0.00
Day 14	MD	147.87	30.13	22.03	115.50	-72.67
	p-value	0.00	0.00	0.00	0.02	0.00

Table 9. Mean Difference (MD) between tests and controls of the effects of combined extracts of *Camellia sinensis, Parquetina nigrescens* and *Telfairia occidentalis* leave extracts on functional iron parameters pre-cobalt 60 irradiation exposure

Day interval		TIBC	FERRITIN	STFr	IronFe	CRP
Day 0	MD	-18.50	1.37	-0.17	-13.77	1.33
	p-value	0.56	0.13	0.95	0.59	0.73
Day 3	MD	49.07	6.17	3.87	20.53	-60.33
	p-value	0.01	0.00	0.03	0.19	0.01
Day 9	MD	76.87	15.77	11.90	84.87	-29.33
	p-value	0.03	0.00	0.00	0.02	0.01
Day 14	MD	158.77	32.63	22.83	70.67	-44.67
	p-value	0.00	0.00	0.00	0.00	0.01

3.2 Post-irradiation Groups

For *C. sinensis*, the mean difference between controls and treated groups was significant from days 3 to 14 all the iron indices and CRP. p-values were 0.00, 0.00, and 0.00 for TIBC, 0.00, 0.00 0.00 for Ferritin , 0.01, 0.00 and 0.00 for STFr, 0.02, 0.00 and 0.00 for iron and 0.01, 0.01 and 0.00 for CRP, Table 6.

For *P. nigrescens*, the mean difference between controls and treated groups was significant fro days 3 to 14 for all the indices and CRP. p-values 0.000, 0.00 and 0.00 for TIBC, 0.00, 0.00 and 0.00 for Ferritin, 0.00, 0.00 and 0.00 for STFr, 0.01, 0.00 and 0.00 for Iron and 0.04, 0.00 and 0.00 for CRP, Table 7.

For *T. occidentalis*, the mean difference between controls and treated groups was significant from days 3 to 14 for all iron indices and CRP. p-values were 0.04,0.00 and 0.00 for TIBC, 0.00, 0.00 and 0.00 for Ferritin, 0.01, 0.00 and 0.00 for STFr, 0.01, 0.00 and 0.02 for Iron and 0.00, 0.00 and 0.00 for CRP, Table 8.

With combined extracts, the mean difference between controls and treated groups was significant for from days 3 to 14 all the indices except iron which became significant from day 9 to 14. For TIBC, p-values were 0.01, 0.03 and 0.00, for Ferritin, p-values were 0.00, 0.00 and 0.00, for STFr, p-values were 0.03, 0.00 and 0.00 and for CRP, p-values were 0.01, 0.01 and 0.01 while for iron it was 0.02 and 0.00, Table 9.

4. DISCUSSION

In this study, functional iron assessment of all the groups revealed statistically significant mean difference in total iron binding capacity, ferritin and soluble transferrin receptor for all the plant extracts**.** These findings clearly delineate that the plants can activate and enhance iron metabolism for subsequent mobilization and incorporation into developing red cells thereby, potentiating erythropoietic cell line differentiation. This may probably be a simple explanation of the mechanisms of the erythropoietic properties previous investigators ascribed to these plants. Interestingly, mean difference of serum iron assay for the groups pre and post irradiation were found to increase significantly with better iron enhancing potential observed with *T. occidentalis and P. nigrescens* treated groups.

The picture of the results here indicated that *T. occidentalis and P. nigrescens* consistently enhanced stored iron which is metabolically inactive and equally enhanced supply of adequate iron to the erythroid marrow. This finding revealed that *C. sinensis* is not a good candidate to use in case of iron deficiency state probably due to the fact that catechins in *C. sinensis* was believed to affect iron absorption, particularly in groups at risk of iron deficiency [39,40], although *C. sinensis* effects on other ions are poorly understood. It could be argued that haemoglobin and some haemopoietic cell lines enhancing effects of *C. sinensis* could be probably due to the fact that its ingestion over a long period does not affect the apparent absorption of copper and also increases that of manganese which are essential minerals for erythropoiesis. However, catechin intake was reported not to affect the plasma concentration of variety of metal ions [40]. In contrast, green tea catechins have been reported to have the potential to affect absorption and metabolism of ions because flavonoids interact with a variety of metal ions [40] (This is beyond the scope of this study because it was stated in the objectives, however, isolation and characterization of extract could be conducted which could be included as part of recommendation).

Worthy of note too is the iron enhancing effects achieved when administered pre-irradiation comparatively between pre and post irradiation. This could be assumed to collaborate the assertion that pathophysiology of anaemia in cancer patients on radiotherapy is multifactorial, but predominantly could be as a result of erythropoietin (EPO) deficiency, iron deficiency and hyporesponsiveness to action of erythropoietin.

Mean difference of serum CRP assay for all the groups both pre and post irradiation phases, followed the same statistical differences with iron indices. This finding on CRP is tangential to the previous claims of previous investigators on the anti-inflammatory properties of these plants. It could mean that some of the inflammatory cytokines were down regulated by these plants. This finding of anti-inflammatory properties is gratifyingly consistent with the previous reports, of Goel et al. [41]; Jagetia et al. [42], that several botanicals demonstrate anti-inflammatory properties. However, none of the researches have considered CRP as a good candidate/marker of inflammatory response assessment in radiation-induced haemopoietic syndrome.

5. CONCLUSION

In addition to the recognized health benefits on normal physiology of the body, *P. nigrescens, C. sinensis* and *T. occidentalis* appear to have potential in providing alternative, rapidly absorbed, non-toxic, good dose reduction factor to act through multiple mechanisms to ameliorate radiotherapy-induced haemopoietic syndrome in cancer patients against the administration of cytokines, blood and blood products transfusion, bone marrow and stem cell transplantation.

The extracts also displayed demonstrable synergistic activity pre-irradiation than in the post-irradiation period.

6. RECOMMENDATIONS

Ameliorative measures against radiation injuries are considered as necessary and urgent but investigations along this line have proceeded slowly and with considerable difficulties. The following recommendations are made from this study: These plants can be put on clinical trials for their effective pharmaceutical use as biological agents in complementary and alternative medicine as antidote to acute radiation syndrome; preclinical work is necessary on non-human primates (NHPs) to establish a good understanding of mechanistic knowledge of efficacy and drug pharmacokinetics since human efficacy trials are with a very stringent, possibly more difficult, approval pathway; and further study on the usefulness of the extracts on patients with renal pathology on dialysis and who are also on routine iron sucrose and erythropoietin regimen can be undertaken.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study and animals used for the experiment were approved by Animal Ethics Committees of University of Ilorin, Nigeria and Ethics and Scientific Committees of the Institute of Endemic Diseases, University of Khartoum during their sitting on 31st of March, 2015 and captured in

the Faculty Board Meeting reference number 4/2015 Minute 1 of 10/04/2015.

All experimental protocols were in compliance with University of Ilorin Ethics Committee on Research in Animals as well as European Union directive 2010/63/EU guidelines for handling animals used for scientific purposes.

ACKNOWLEDGEMENT

The authors acknowledged the technical staff of Department of Pharmacognosy and Drug Development, University of Ilorin, Ilorin; and Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria; and the technologists at Central Science Laboratories, Obafemi Awolowo University, Ile-Ife, Nigeria for their roles in the collection, and extraction of plant materials. They also acknowledged the technical staff of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin for the animal handling and care; and Department of Medical Laboratory Services (Haematology, Chemical Pathology, and Histopathology Units), University of Ilorin Teaching Hospital, Ilorin for samples analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Metzgeroth G, Hastka J. Iron deficiency anemia and anemia of chronic disorders. Internist (Berl). 2015;56:978–988.
2. Khan MA, Hill RP, Van Dyk J. Partial volume rat lung irradiation: An evaluation of early DNA damage. Int J Radiat Oncol Biol Phys. 1998;40:467–476.
3. Mukherjee D, Coates PJ, Lorimore SA, et al. The *in vivo* expression of radiation-induced chromosomal instability has an inflammatory mechanism. Radiat Res. 2012;177:18–24.
4. Kim K, McBride WH. Modifying radiation damage. Curr Drug Targets. 2010;11: 1352–1365.
5. Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: A review. Radiat Res. 2012;178:505–523.
6. Cazzola M, Ponchio L, de Benedetti F, et al. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood. 1996;87:4824–4830.
7. Castell JV, Gomez-Lechon MJ, David M, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett. 1989;242: 237–239.
8. Jelkmann W. Proinflammatory cytokines lowering erythropoietin production. J. Interferon Cytokine Res. 1998;18:555–559.
9. Rodriguez RM, Corwin HL, Gettinger A, Corwin MJ, Gubler D, Pearl RG. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. J Crit Care. 2001;16:36–41.
10. Taniguchi S, Dai CH, Price JO, Krantz SB. Interferon gamma downregulates stem cell factor and erythropoietin receptors but not insulin-like growth factor-I receptors in human erythroid colony-forming cells. Blood. 1997;90:2244–2252.
11. Wang CQ, Udupa KB, Lipschitz DA. Interferon-gamma exerts its negative regulatory effect primarily on the earliest stages of murine erythroid progenitor cell development. J. Cell. Physiol. 1995;162: 134–138.
12. Maciejewski JP, Selleri C, Sato T, et al. Nitric oxide suppression of human hematopoiesis *in vitro*. Contribution to inhibitory action of interferon-gamma and tumor necrosis factor-alpha. J. Clin. Invest. 1995;96:1085–1092.
13. Libregts SF, Gutierrez L, de Bruin AM, et al. Chronic IFN-gamma production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. Blood. 2011;118:2578–2588.
14. Feng Q, Kumagai T, Torii Y, Nakamura Y, Osawa T, Uchida K. Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. Free Radical Res. 2001;35:779-788.
15. Kondo T, Ohta T, Igura K, Hara Y, Kaji D. Tea catechins inhibit angiogenesis endothelial cell growth, migration *in vitro*, measured by human and tube formation through inhibition of VEGF receptor binding. Cancer Lett. 2002;180:139-144.
16. Sakata R, Ueno T, Nakamura T, Sakamoto M, Torimura T, Sata M. Green tea polyphenols epigallocatechin-3-gallate inhibits platelet-derived growth factor-induced proliferation of human hepatic

stellate cell line LI90. J Hepatol. 2004;40: 52-59.
17. Park H, Ko S, Kim J, Kim S. Effects of green tea extracts and polyphenols on the proliferation and activity of bone cells. J Bone Miner Res. 2003;18:S342.
18. Brown AL, Lane J, Holyoak C, Nicol B, Mayes AE, Dadd T. Health effects of green tea catechins in overweight and obese men: A randomised controlled cross-over trial. Br. J. Nutr. 2011;106(12): 1880-1889.
19. Gbile ZO. Ethnobotany, taxonomy and conservation of medicinal plant in the state of medicinal plant research in Nigeria. 1986;19.
20. Dina OA, Adedapo AA, Oyimloye OP, Saba AB. Effects of *Telfairia occidentalis* extracts on experimentally induced anemia in wistar rats. African Journal of Biomedical Science Resources. 2006;3: 181-183.
21. Oluwole FS, Falade AO, Ogundipe OO. Antiinflammatory effect of some Common Nigerian vegetables. Nig. J. Physiol. Sci. 2003;18(1-2):35-38.
22. Ajayi OI, Ajayi TC, Omokaro ED, Halim NKD. Erythropoietic value of *T. occidentalis* in rabbit. A preliminary study. Nig. J. Physiol. Sci. 2000;16(1-2):1-3.
23. Eseyin OA, Igboasoiyi AC, Oforah E, Ching P, Okoli BC. Effect of extract of *T. occidentalis* leaves on some biochemical parameters in rat. Glob. J. Pure and Applied Sciences. 2005;11(1):85-87.
24. Eseyin OA, Igboasoiyi AC, Oforah E, Nkop, Agboke A. Hypoglycemic activity of *T. occidentalis* in rats. J. Pharm. and Bioresources. 2005;2(1):36-42.
25. Ekpenyong CE, Akpan EE, Udoh NS. Phytochemistry and toxicity studies of *Telfairia occidentalis* aqueous leaves extract on liver biochemical indices in wistar rats. American Journal of Medicine and Medical Sciences. 2012;2(5):103-110, 105.
26. Schlage C. Medical plants of the Wasambas (Tanzania): Documentation and ethnopharmaological evaluation. Plant Biology. 2002;2:83-92.
27. Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A. An ethnopharmacological survey and *in vitro* confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J. Ethnopharmacol. 2009;125(3): 393–403.
28. Imaga NOA, Gbenle GO, Okochi VI, Adenekan SO, Edeoghon SO, Kehinde M, Bamiro SB, Ajiboye A, Obinna A. Antisickling and toxicological profiles of leaf and stem of *Parquetina nigrescens* L. Journal of Medicinal Plants Research. 2010;4(8):639-643.
29. Agbor AG, Odetola AA. Haematological studies of *Parquetina nigrescens* on haemorrhagic anaemic rats. Afr. J. Med. Sci. 2001;30:105-109.
30. Erah OP, Asonye CC, Okhamafe OA. Response of *Trypanosoma brucei*-induced anaemia to a commercial herbal preparation. AJB. 2003;2:307-311.
31. Kade IJ, Kotila OO, Ayeleso AO, Olaleye AA, Olawoye TL. Antisickling properties of *Parquetina nigrescens*. Biomed. Res. (Alligarh). 2003;14:185-188.
32. Akinyemi OI, Dada EO. Phytochemical screening and antityphoid properties of ethanolic leaf extracts of *Parquetina nigrescens*. ARPN Journal of Agricultural and Biological Science. 2013;8(10):732-739.
33. Akinyemi OI, Dada EO. *In vivo* antityphoid activities and proximate analysis of ethanolic leaf extracts of *Parquetina nigrescens*. Journal of Pharmacy and Biological Sciences. 2014;9(5):115-123.
34. Olatubosun LO, Biliaminu SA, Lawal SA, Olalere FD, Raheem RA, Mohammed AO, Rasheed T. Combined synergistic effects of aqueous extracts of extracts of *Parquetina nigrescens*, *Camellia sinensis* and *Telfaria occidentalis* on bone marrow haemopoietic multipotent stem cells proliferation in irradiated guinea pigs. International Journal of Sciences: Basic and Applied Research (IJSBAR). 2014;15(1):139-150.
35. Sofowora A. Phytochemical screening: Medicinal plants and traditional medicine in Africa. 3rd Ed. Spectrum; 2008.
36. Evans WC. Trease and Evans pharmacognosy. 16th Ed. Elsievier Books Limited, Ibadan, Nigeria. 2009;199-204.
37. Harris Ronald. Rehabilitation in Hemiplagia, Occupational Therapy, 8-11.
38. Malene Nelson, Poulter J. Impact of tea drinking on iron status in the UK: A review. J. Hum Nutr Diet. 2004;17(1):43-54.
39. Samman S, Sandström B, Toft MB, Bukhave K, Jensen M, Sørensen SS,

Hansen M. Green tea or rosemary extract added to foods reduces nonheme-iron absorption. Am J Clin Nutr. 2001;73(3): 607-12.

40. Jia-bin Deng, Chun-bang Ding, Li Zhang, Rui-wu Yang, Yong-hong Zhou. Authentication of three related herbal species (Curcuma) by DNA barcoding. Journal of Medicinal Plants Research. 2011;5(28):6401-6406.

41. Goel AK, Kulshreshtha DK, Dubey MP, Rajendran SM. Screening of Indian plants for biological activity: Part XVI. Indian Jounal of Experimental Biology. 2002;40(7):812-27.

42. Jagetia GC, Baliga MS. Influence of the leaf extract of *Mentha arvensis* Linn. (mint) on the survival of mice exposed to different doses of gamma radiation. Strahlenther. Onkol; 2002.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/52698