Original Research Article

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.

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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 inavailability 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 sequell 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 (4.0Gy) whole-body gamma-irradiation under general anaesthesia, using a Co^{60} therapy unit as a source. Groups A1, A2, A3 and A4 had 1,400mg/kg C. sinensis, 400mg/kg P. negrescens, 3,500mg/kg T. occidentalis and Combined dose (1500mg/kg C. sinensis +4000mg/kg P. negrescens +3500mg/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 throughtout the 14 days of the study. Group C were not given any treatment but also had irradiation.

Results: Total Iron Binding Capacity, Feritin, Serum Transferin 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.

Key Words**:** Functional Iron Indices, Plant Extracts, Irradiation.

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 inflamations 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 transferin expression in the liver, thus reducing the serum's capacity to transport iron.[7] Erythropoeitin (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 transferin saturation, there is non-regenerative normocytic or microcytic anaemia.

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 apetite, 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 ~~aqeous~~ aqueous extracts of Parquetina nigrescens, Telfairia occidentalis and Camellia sinensis in single and combined doses since they have been reported in our previous study to have synergistic effects on bone marrow haemopoeitic multipotent stem cells differentiation and proliferation post irradiation[14]. We also assessed the level of C - reactive protein (CRP) as an indication of on-going inflammatory process.

Comment [u1]: Include the ethnopharmacological information of these plants.

MATERIALS AND METHODS:

Study design:

This was an experimental and interventional study.

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* 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.

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.

Preliminary Phytochemical Screening:

The phytochemical constituent of the aqueous extracts were determined using standard procedures as described previously (Sofowora, 2008; Evans, 2009). The extracts were tested for the presences or absence of saponins, tannins, alkaloids, anthraquinones, cardiac glycosides, flavonoids and terpenoids.

Animal Care:

Comment [u2]: How did you authenticate the purity of the product

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Comment [u3]: You would require an bath temperature of 100 degree cel. for the aqueous extract to evaporate. Check your protocol.

Comment [u4]: Make and model?

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 ($34 \times 47 \times 18$ 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 ($60\% \pm 10\%$) 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. 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.

Animal Grouping for the Study:

Fifty-Five young male guinea-pigs approximately 450 gram in weight obtained from the animal house, 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 groups C served as control.

Comment [u5]: Confusing. Use a table to explain your grouping clearly.

Administration of Extracts:

Groups A1, A2, A3 and A4 had 1,400mg/kg C. sinensis, 400mg/kg P. negrescens, 3,500mg/kg T. occidentalis and Combined dose (1500mg/kg C. sinensis +4000mg/kg P. negrescens +3500mg/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 throughtout the 14 days of the study. Group C were not given any treatment but also had irradiation.

Comment [u6]: What was the criteria for dose fixation. Why 400 mg/kg of P. negrescens in individual dose and 4000 mg/kg in combined dose.

Comment [u7]: 8000 mg/kg combined dose to guinea pigs looks something huge. If you extrapolate this to human equivalent dose, it would be impossible for a normal human to take as a single dose. On what strategy the combined dose was planned.

Method and Dose of Irradiation:

Irradiation was done at University College Hospital Ibadan, Oyo State, Nigeria by the method described by Harris (1960). After general anesthesia using intra-muscular ketamine 5mg/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 (4.0Gy) 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 3Gy/1.53minute.

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 descired by Malene, et al (2004) 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.

After-Care of the Irradiated Animals:

To minimize the two major complications enumerated by Harris (1960) 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.

Functional Iron Assessment:

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:

Unsaturated Iron-Binding Capacity (UIBC)

Iron level + UIBC = TIBC (ug/dl)

SI Unit Conversion µg/dl × 0.179=µmol/l.

Soluble Transferin Receptor Assay:

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

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.

SCIENTIFIC AND ETHICAL CONSIDERATION:

The study and animals used for the experiment were approved by Animal Ethics Committees of University of Ilorin, Nigeria and Ethics & 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.

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 significancy considered at $p<0.05$.

Comment [u8]: Values are not expressed with standard deviation. Give SD next to your mean value in all the tables.

RESULTS:

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 1.

For P. nigrescens, the mean difference between controls and treated groups was significant fro 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 2.

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 3.

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 4.

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 5.

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 6.

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 7.

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 8.

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 Telfairia and Parquetina treated groups. The picture of the results here indicated that Telfairia and Parquetina consistently enhanced stored iron which is metabolically inactive and equally enhanced supply of adequate iron to the erythroid marrow. This finding revealed that camellia sinensis is not a good candidate to use in case of iron deficiency state probably due to the fact that catechins in camellia sinensis was believed to affect iron absorption, particularly in groups at risk of iron deficiency[15,16], although camellia sinensis's effects on other ions are poorly understood. It could be argued that haemoglobin and some haemopoietic cell lines enhancing effects of camellia 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[16]. 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[16].

Comment [u9]: What could be the possible bioactive ingredients in your study. Discuss with relevant references.

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[17-19] of Goel et al., 2002; Jagetia et al., 2002 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.

Comment [u10]: Discuss on the recommended human equivalent dose (HED) from your study.

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.

RECOMMENDATIONS:

Ameliorative measures aginst 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 futher 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.

CONFLICT OF INTERESTS:

The authors declared no conflict of interests.

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Table 1: 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 2: 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 3: 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 4: 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 5: 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 6: 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

Table 7: 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 8: 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	FERITIN	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