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Functional Iron Indices and Highly Sensitive CRP Post Single Dose Irradiation with Cobalt⁶⁰ and the Ameliorating Effects of Single and Combined Doses of Aqueous Extracts of *Parquetina nigrescens*, *Camellia sinensis* and *Telfairia occidentalis* in Guinea Pigs.

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 Co⁶⁰ 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

27 continued throughout the 14 days of the study. Group C were not given any treatment but also had
28 irradiation.

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

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

34

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

36 INTRODUCTION

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

48 There are multiple players in the pathophysiology of AI: The excessive production of inflammatory
49 mediators diverts iron to the mononuclear phagocyte system (MPS) rendering it relatively
50 unavailable for erythroid progenitors.⁶ Hepcidin anti-microbial peptide (HAMP), one of such
51 inflammatory mediators is the hormonal negative-feedback regulator of serum iron, as it limits iron-
52 fluxes to the circulation. It is release from the liver due to inflammation caused by irradiation.

53 Hepcidin blocks iron absorption and inhibits its recycling from senescent red blood cells. Other
54 inflammatory mediators released upon liver injury are IL 1 and 6R. IL-6 and other pro-inflammatory
55 cytokines result in a downregulation of transferrin expression in the liver, thus reducing the serum's
56 capacity to transport iron.⁷ Erythropoietin (EPO) production in the kidney is also inhibited by
57 inflammatory mediators such as tumor necrosis factor (TNF) and IL-1.^{8,9} Aside reduced production
58 of EPO, there is downregulation of the EPO receptor on erythroid cells by interferon (IFN)- γ ,¹⁰
59 another inflammatory mediator. Tumour Necrosis Factor (TNF), IL-1, IFN- γ , and other reactive
60 intermediates inhibits the proliferation and differentiation of erythroid progenitors.^{11,12,13}
61 The combined effect of Hepcidin and these inflammatory mediators results into functional iron
62 deficiency, whereby there is reduced availability of iron but with normal or increased iron reserve,
63 while EPO deficiency further inhibits incorporation of iron into erythroid progenitor, Burst Forming
64 Unit Erythroid (BFUe). With low transferrin saturation, there is non-regenerative normocytic or
65 microcytic anaemia.

66 Several recommendations have been presented regarding how to cope with radiation effects among
67 which are drinking of at least 8 ounce glasses of water per day for diarrhea and dehydration, taken of
68 tarts fruits or fruit-flavored sourballs in case of change of taste, eating small frequent meals to
69 reduce nausea and vomiting and for patients with loss of appetite, rinsing of mouth with water before
70 meals for patients with dry mouth and so on. Measures to ameliorate the hypoferremic condition
71 promoted by inflammation will be of immense benefit to patients after radiotherapy or radiation
72 injury also. It was in the light of this that we decided to assess functional iron indices post irradiation
73 and evaluate the potential benefit of the aqueous extracts of *Parquetina nigrescens*, *Telfairia*
74 *occidentalis* and *Camellia sinensis* in single and combined doses since they have been reported in
75 our previous study to have synergistic effects on bone marrow haemopoietic multipotent stem cells
76 differentiation and proliferation post irradiation¹⁴. We also assessed the level of C - reactive protein
77 (CRP) as an indication of on-going inflammatory process.

78 **MATERIALS AND METHODS:**

79 **Study design:**

80 This was an experimental and interventional study.

81 **Collection and Identification of the plant material:**

82 Fresh leaves of *Parquetina nigrescens* and *Telfairia occidentalis* were collected from University of
83 Ilorin, Nigeria Plant Garden while a refined product of *Camellia sinensis* was purchased from
84 pharmaceutical premises in Ilorin, Nigeria. The plants were identified and authenticated by a
85 taxonomist at the Department of Plant Biology, University of Ilorin, Nigeria. *Parquetina nigrescens*
86 was given Serial Number 876 and Ledger Number 67 while *Telfairia occidentalis* was given Serial
87 Number 959 and Ledger Number 150. Thereafter, collected samples were deposited in the
88 herbarium of the institution for future references.

89

90 **Processing of the Plants' Extracts:**

91 Four hundred grams and 350 g of the powdered leaves of *Telfairia occidentalis* and *Parquetina*
92 *nigrescens* respectively were each soaked in distilled water in a closable container. The finished
93 product (fine granules) of *Camellia sinensis* was also weighed and soaked in distilled water. These
94 were shaken for about 5 minutes and left to extract by means of maceration (shaking the mixture
95 intermittently) at 28 °C for 72 hours. The mixtures were filtered into a porcelain crucible using a fine
96 mesh. The supernatant was concentrated below 40 °C using rotary evaporator and then freeze-dried.
97 The extract was stored at 4 °C in freeze-dried form.

98 **Preliminary Phytochemical Screening:**

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

103 **Animal Care:**

104 All Animals were kept in the animal house of the Toxicology Unit, Department of Pharmacology,
105 Faculty of Pharmacy, University of Ilorin, Nigeria. The animals were kept in plastic cages
106 ($34 \times 47 \times 18 \text{ cm}^3$) in an air conditioned environment with one Guinea-pig in each cage. Animals
107 maintained at room temperature of $(25 \pm 2) ^\circ\text{C}$ of relative humidity ($60\% \pm 10\%$) under 12 hour
108 night and light cycle. They had free access to standard pellets as basal diet and water ad libitum.
109 Animals were habituated to laboratory conditions for two weeks prior to experimental protocol to
110 minimize if any of non-specific stress. All experimental protocols were in compliance with
111 University of Ilorin Ethics Committee on Research in Animals as well as European Union directive
112 2010/63/EU guidelines for handling animals used for scientific purposes.

113 **Animal Grouping for the Study:**

114 Fifty-Five young male guinea-pigs approximately 450 gram in weight obtained from the animal
115 house, LAUTECH College of Medicine Osogbo, Osun-State, Nigeria were recruited and thirty were
116 randomly assigned to 3 groups (A, B and C) for the study. Groups A and B were further divided into
117 4 (A1-4 and B1-4) with 3 animals ($n=3$) per group. Three guinea-pigs were also assigned to group C.
118 Groups A and B belonged to Pre and post-irradiation groups while groups C served as control.

119 **Administration of Extracts:**

120 Groups A1, A2, A3 and A4 had 1,400mg/kg *C. sinensis*, 400mg/kg *P. negrescens*, 3,500mg/kg *T.*
121 *occidentalis* and Combined dose (1500mg/kg *C. sinensis* +4000mg/kg *P. negrescens* +3500mg/kg *T.*
122 *occidentalis*) respectively twice daily 72 hours prior to irradiation and continued throughout the 14
123 days of the study. Groups B1, B2, B3 and B4 had similar treatment but commenced 24 hours after
124 exposure to irradiation and likewise continued throughout the 14 days of the study. Group C were
125 not given any treatment but also had irradiation.

126 **Method and Dose of Irradiation:**

127 Irradiation was done at University College Hospital Ibadan, Oyo State, Nigeria by the method
128 described by Harris (1960). After general anesthesia using intra-muscular ketamine 5mg/Kg body
129 weight plus 1 mg Atropine, each guinea-pig was placed in a cotton-gauze bag and positioned lying

130 on the side. Each animal was given 400r (4.0Gy) whole-body gamma-irradiation under general
131 anaesthesia, using a Co⁶⁰ therapy unit as a source. The radiation technique is Source Skin Distance
132 (SSD) at the depth of 4cm and dose rate of 3Gy/1.53minute.

133 **Sample collection:**

134 2 milliliters of venous blood was collected aseptically from the Lateral Saphenous vein from each
135 animal on days 0 (day of irradiation), 3, 9 and 14 using the method descired by Malene, et al (2004)
136 according to protocols approved by the Danish Animal Experimentation Inspectorate under the
137 Ministry of Food, Agriculture and Fisheries. The samples were dispensed into bottles containing
138 EDTA and analyzed immediately.

139 **After-Care of the Irradiated Animals:**

140 To minimize the two major complications enumerated by Harris (1960) i.e. the danger of internal
141 haemorrhage from minor trauma and the risk of infection, resulting from the effects of irradiation on
142 haemopoietic tissues, each irradiated animal were kept in a separate cage and excessive handling
143 avoided until it was due for sacrifice. Each animal was adequately fed and given adequate supply of
144 water.

145 **Functional Iron Assessment:**

146 **Serum Iron & Total Iron Binding Capacity:**

147 Serum iron assay and Total iron binding capacity were determined together by colorimetric method
148 using the commercially prepared Pointe Scientific, Inc iron/TIBC reagent, 2016.

149 **Serum Ferritin Assay** was assessed using commercial kits supplied by Alpcó:

150 **Unsaturated Iron-Binding Capacity (UIBC)**

151 Iron level + UIBC = TIBC (ug/dl)

152 SI Unit Conversion $\mu\text{g/dl} \times 0.179 = \mu\text{mol/l}$.

153 **Soluble Transferin Receptor Assay:**

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

156 **C-Reactive Protein:**

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

159 **SCIENTIFIC AND ETHICAL CONSIDERATION:**

160 The study and animals used for the experiment were approved by Animal Ethics Committees of
161 University of Ilorin, Nigeria and Ethics & Scientific Committees of the Institute of Endemic
162 Diseases, University of Khartoum during their sitting on 31st of March, 2015 and captured in the
163 Faculty Board Meeting reference number 4/2015 Minute 1 of 10/04/2015.

164 **DATA ANALYSIS:**

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

168 **RESULTS:**

169 **Pre-irradiation groups:**

170 For *C. sinensis*, the mean difference between controls and treated groups was significant from days 3
171 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
172 0.00, 0.00, 0.00) while it only became significant on day 14 for serum iron (p-values 0.00), Table 1.

173 For *P. nigrescens*, the mean difference between controls and treated groups was significant fro days 3
174 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,
175 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.

176 For *T. occidentalis*, the mean difference between controls and treated groups was significant from
177 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
178 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
179 TIBC (p-values 0.00, 0.01), Table 3.

180 With combined extracts, the mean difference between controls and treated groups was significant for
181 all the indices except iron. For TIBC, p-values were 0.05, 0.00 and 0.00, for Ferritin, p-values were

182 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
183 and 0.00 while for iron it was 0.01 and 0.00, Table 4.

184 **Post-irradiation groups:**

185 For *C. sinensis*, the mean difference between controls and treated groups was significant from days 3
186 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
187 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,
188 Table 5.

189 For *P. nigrescens*, the mean difference between controls and treated groups was significant fro days 3
190 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
191 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,
192 Table 6.

193 For *T. occidentalis*, the mean difference between controls and treated groups was significant from
194 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
195 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
196 for CRP, Table 7.

197 With combined extracts, the mean difference between controls and treated groups was significant for
198 from days 3 to 14 all the indices except iron which became significant from day 9 to 14. For TIBC,
199 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
200 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
201 and 0.00, Table 8.

202 **DISCUSSION:**

203 In this study, functional iron assessment of all the groups revealed statistically significant mean
204 difference in total iron binding capacity, ferritin and soluble transferrin receptor for all the plant
205 extracts. These findings clearly delineate that the plants can activate and enhance iron metabolism
206 for subsequent mobilization and incorporation into developing red cells thereby, potentiating
207 erythropoietic cell line differentiation. This may probably be a simple explanation of the

208 mechanisms of the erythropoietic properties previous investigators ascribed to these plants.
209 Interestingly, mean difference of serum iron assay for the groups pre and post irradiation were found
210 to increase significantly with better iron enhancing potential observed with Telfairia and Parquetina
211 treated groups. The picture of the results here indicated that Telfairia and Parquetina consistently
212 enhanced stored iron which is metabolically inactive and equally enhanced supply of adequate iron
213 to the erythroid marrow. This finding revealed that camellia sinensis is not a good candidate to use in
214 case of iron deficiency state probably due to the fact that catechins in camellia sinensis was believed
215 to affect iron absorption, particularly in groups at risk of iron deficiency^{15,16}, although camellia
216 sinensis's effects on other ions are poorly understood. It could be argued that haemoglobin and some
217 haemopoietic cell lines enhancing effects of camellia sinensis could be probably due to the fact that
218 its ingestion over a long period does not affect the apparent absorption of copper and also increases
219 that of manganese which are essential minerals for erythropoiesis. However, catechin intake was
220 reported not to affect the plasma concentration of variety of metal ions¹⁶. In contrast, green tea
221 catechins have been reported to have the potential to affect absorption and metabolism of ions
222 because flavonoids interact with a variety of metal ions¹⁶.

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

228 Mean difference of serum CRP assay for all the groups both pre and post irradiation phases,
229 followed the same statistical differences with iron indices. This finding on CRP is tangential to the
230 previous claims of previous investigators on the anti-inflammatory properties of these plants. It
231 could mean that some of the inflammatory cytokines were down regulated by these plants. This
232 finding of anti-inflammatory properties is gratifyingly consistent with the previous reports¹⁷⁻¹⁹ of
233 Goel et al., 2002; Jagetia et al., 2002 that several botanicals demonstrate anti-inflammatory

234 properties. However, none of the researches have considered CRP as a good candidate/marker of
235 inflammatory response assessment in radiation-induced haemopoietic syndrome.

236 **CONCLUSION:**

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

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

244 **RECOMMENDATIONS:**

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

255 **CONFLICT OF INTERESTS:**

256 The authors declared no conflict of interests.

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313

314 **Table 1: Mean Difference (MD) between tests and controls of the effects of *Camellia sinensis***
 315 **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 |

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318 **Table 2: Mean Difference (MD) between tests and controls of the effects of *Parquetina***
 319 ***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 |

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325 **Table 3: Mean Difference (MD) between tests and controls of the effects of *Telfairia***
326 ***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 |

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329 **Table 4: Mean Difference (MD) between tests and controls of the effects of combined extracts**
330 **of *Camellia sinensis*, *Parquetina nigrescens* and *Telfairia occidentalis* leave extracts on**
331 **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 |

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339 **Table 5: Mean Difference (MD) between tests and controls of the effects of *Camellia sinensis***
340 **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 |

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342 **Table 6: Mean Difference (MD) between tests and controls of the effects of *Parquetina***
343 ***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 |

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349 **Table 7: Mean Difference (MD) between tests and controls of the effects of *Telfairia***
 350 ***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 |

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352 **Table 8: Mean Difference (MD) between tests and controls of the effects of combined extracts**
 353 **of *Camellia sinensis*, *Parquetina nigrescens* and *Telfairia occidentalis* leave extracts on**
 354 **functional iron parameters pre-cobalt 60 irradiation exposure.**

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| 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 |

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