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Research Article

Ranferon-12 Tonic Mitigates Haematological Abnormalities in Cyclophosphamide-treated Rats

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ABSTRACT

Cyclophosphamide (CPA) has anticancer property with many side-effects including haematotoxicity while ranferon-12 tonic (RFT) possesses haematinic effect. This study investigated the mitigating effects of RFT on haematotoxicity in CPA-treated rats. Twenty-four animals were grouped into four (n=6) and treated orally as indicated: Group A: (Control) received 0.4 mL of physiological saline (PS) for 7 consecutive days; Group B: (CPA) received 0.4 mL of PS for 7 days followed by a single intraperitoneal dose of CPA (200 mg/kg) on the 7th day; Group C: (RFT) received RFT at 0.029 mL/kg for 7 days; Group D: (RFT+CPA) received RFT at 0.029 mL/kg for 7 days followed by a single intraperitoneal dose of CPA on the 7th day. Twenty-four hours after the final treatment, the animals were weighed, anaesthetised and sacrificed. Blood was obtained via cardiac puncture and transferred to EDTA and plain tubes for further analysis. Result showed that a single dose of CPA (200 mg/kg) significantly ($p < 0.05$) reduced the level of white blood cells, haemoglobin, red blood cells, packed cell volume, and platelet (values >36%). Additionally, the body temperature was significantly ($p<0.05$) elevated by 5.1 %. Furthermore, decreased levels of superoxide dismutase and catalase with a concomitant rise in MDA content were observed. All these alterations were mitigated; to some extent, in animals pre-treated with RFT prior to CPA-administration. The study suggests that RFT could mitigate haematotoxicity in CPA-administered rats by suppressing oxidative stress via modulation of haematopoietic factors, which in turn promotes haematopoiesis.

Keywords *Ranferon-12 tonic, Cyclophosphamide, Haematotoxicity, Oxidative stress, Haematopoietic factor, Haematopoiesis*

INTRODUCTION

Cyclophosphamide (CPA) is a common anticancer agent often used to improve the quality of a cancer patient's life. However, it is limited due to unpleasant side effects, e.g., haematotoxicity (Ferguson and Pearson, 1996; Iqubal *et al.,* 2020; Kameo *et al.,* 2021). CPA-induced haematotoxicity is a prevalent side effect that corresponds to haematopoietic impairment, which may lead to reduced levels of red blood cells (RBCs), decreased haematocrit, reduced haemoglobin counts (anaemia), leukopenia, thrombocytopenia, haemorrhage, or immunosuppression (Fraiser *et al.,* 1991; Kameo *et al.,* 2021).

CPA-induced side effects are the product of inflammation and oxidative stress caused by a xenobiotic metabolite (acrolein) via the enzymatic metabolism of CPA (Colvin, 1999; Peng *et al.,* 2022). The present study focuses on side effects, particularly haemototoxicity because it (a) limits dose increment and (b) may jeopardise the continuation of treatment (Testart-Paillet *et al.,* 2007). Handling such a toxic effect would be important for treating patients more effectively (El-Naggar *et al.,* 2015; Hoffman *et al.,* 2005; Ukpo *et al.,* 2013). Thus, the need for specific adjuvant therapy that can mitigate or eliminate the toxic effects of CPA without altering its anticancer potential will greatly benefit CPA treatment strategies.

Ranferon-12 tonic (RFT) is a pure synthetic blood-building tonic that contain haematinic factors for rapid and optimum formation of healthy red blood cells as well as haematopoiesis

(Koury and Ponka, 2004; Mitra *et al.,* 2022). However, its effect on CPA-induced haemototoxicity remains unexplored. Therefore, this study attempts to explore the haematopoietic potential of RFT in mitigating CPA-induced haematotoxicity in Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

Cyclophosphamide (Mumbai, India); Ranferon-12 tonic (Ogun, Nigeria); Ketamine hydrochloride (Gujarat, India); Trichloro acetic acid, thiobarbituric acid, glacial acetic acid, and potassium heptaoxodichromate were procured from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); Disodium hydrogen orthophosphate, sodium hydroxide, hydrochloric acid, and sodium dihydrogen phosphate were products of BDH Company (UK, England).

Experimental model and ethical approval

Wistar male rats weighing 180±20 g were purchased from animal facility at Okuku Campus, University of Cross River State (UNICROSS), Nigeria. The animals were kept in rodent facility at ambient condition with 12-hour day/night cycle and given unlimited access to feed and drinking water. The animals were acclimatised for two weeks prior to experiment. Additionally, Institutional Research and Ethics Committee of the Faculty of Basic Medical Sciences, University of Cross River State (UNICROSS), Okuku Campus, Nigeria (UNICROSS/FBMS/IREC/2022-A08) approved the study in line with the guidelines of Helsinki Declaration (1964), as revised in 2013.

Experimental protocol

Twenty-four male Wistar rats were grouped into four (n=6) and treated orally as indicated: Group A: (Control) received 0.4 mL of physiological saline (PS) for 7 consecutive days; Group B: (CPA) received 0.4 mL of PS for 7 days followed by a single intraperitoneal dose of CPA (200 mg/kg) on the 7th day; Group C: (RFT) received RFT at 0.029 mL/kg for 7 days; Group D: (RFT+CPA) received RFT at 0.029 mL/kg for 7 days followed by a single intraperitoneal dose of CPA on the 7th day. Twenty-four hours after the final treatment, the animals were weighed, anaesthetised (via ketamine; 90 mg/kg) and sacrificed. Blood was obtained via cardiac puncture and transferred to EDTA and plain tubes for further analysis. Blood was collected via cardiac puncture and transferred immediately to two different containers (EDTA and plain tubes) for further analysis. The dose of RF (0.029 mL/kg) is equivalent to 10 mL recommended for a 70 kg adult man, and cyclophosphamide (200 mg/kg) intraperitoneal injection based on literature findings (El-Naggar *et al.,* 2015; Iqubal *et al.,* 2020; Lakshmi *et al.,* 2005).

Determination of body temperature

The body temperature was measured (before and after CPA injection) through the rectal duct using a digital thermometer (KRIS-ALOY, Wuxi Honguang Medical Equipment CO, Ltd. Jiangsu, China).

Determination of haematological indices

Haematological indices were measured by analysing blood samples collected in EDTA tubes for white blood cell (WBC), haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), and platelet (PLT) counts, using automated haematological analyser (Abacus J30, Spectrum Medical Industries Private Ltd. New Delhi, India).

Determination of antioxidant parameters

The whole blood drawn into plain tubes was allowed to clot for an hour and spun for 10 min at 1500 x g. Serum obtained was used for further investigation. According to Buege and Aust (1978), lipid peroxidation was measured by monitoring the rate of thiobarbituric acid reactive substances (TBARS) formation as malondialdehyde (MDA). Additionally, the activities of superoxide dismutase (SOD) and catalase (CAT) were monitored respectively according to the method of Sinha (1972) and Martin *et al.* (1987).

Statistical package

Data obtained from the study were presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests were carried out using Graph Pad Prism software. Data at $p < 0.05$ was considered statistically significance.

RESULTS

Figure 1-5 shows the level of some haematological indices in CPA-treated rats following oral administration of RFT. In Group B (CPA control), a single intraperitoneal dose of CPA (200 mg/kg/rat), after 24 h, significantly ($p < 0.05$) reduce the concentration of red blood cell, haemoglobin, packed cell volume, white blood cell, and platelet respectively as follows: 4.34±0.18 10^6/µl, 8.28±0.34 g/dl, 25.14±1.05 %, 2.85±0.20 10^3/µl and 475.3±45 10^3/µl compared to the control group (7.02±0.20 10^6/µl, 12.87±0.32 g/dl, 39.50±1.10 %, 8.17±0.22 10^3/µl, and 1056.2±50 10^3/µl). These decreases approximately correspond to 38%, 36%, 36%, 65% and 55% respectively. However, animals (RFT+CPA; group C) pre-treated orally for 7 consecutive days with RFT at 0.029 mL/kg prior to CPA administration, increased significantly ($p < 0.05$) the concentration of red blood cell, haemoglobin, packed cell volume, white blood cell, and platelet count (5.84±0.20 10^6/µl, 10.45±0.29 g/dl, 31.95±1.08 %, 4.52±0.21 10^3/µl, and 680.5±40 103/µl) relative to group B (CPA control). These increases approximately correspond to 35%, 26%, 27%, 59% and 43% respectively. Whereas administration of only RFT at 0.029 mL/kg (group D), improved all the haematological parameters studied (7.34±0.22 10^6/µl, 13.42±0.30 g/dl, 41.82±1.15 %, 8.27±0.24 10^3/µl, and 1090.5±48 10^3/µl), relative to normal control (group A).

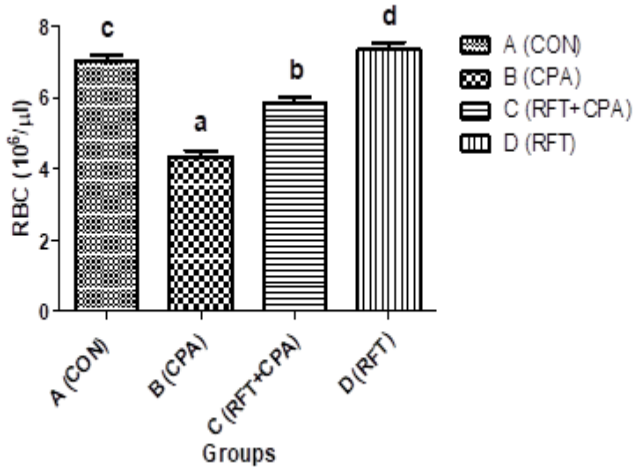

Figure 1. Influence of RFT on Red Blood Cell Count (RBC) in CPA-administered Rats. Data were presented as mean ± SD (n=6). CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic; CON = Control

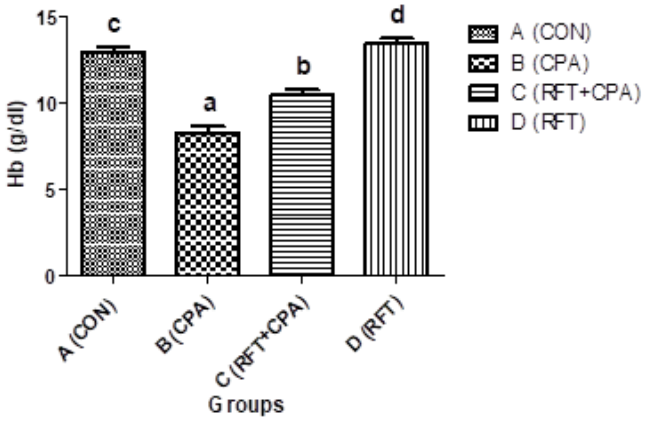

Figure 2. Influence of RFT on Haemoglobin (Hb) Level in CPA-administered Rats. Data were presented as mean ± SD (n=6). CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic; CON = Control

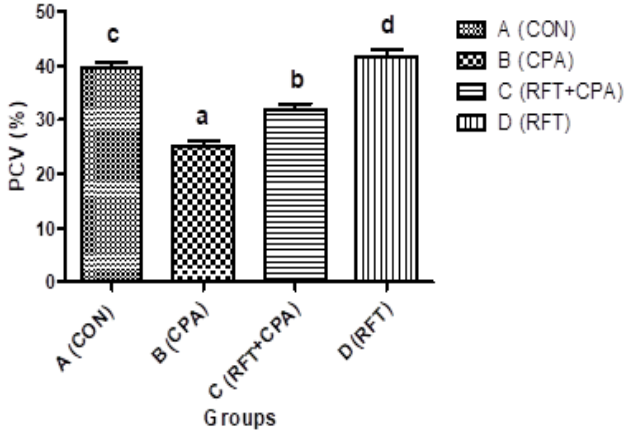

Figure 3. Influence of RFT on Packed Cell Volume (PCV) in CPA-administered Rats. Data were presented as mean ± SD (n=6). CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic; CON = Control

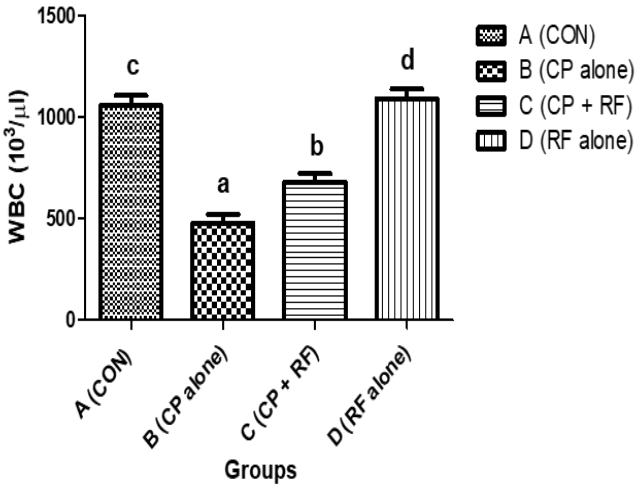

Figure 4. Influence of RFT on White Blood Cell Count (WBC) in CPA-administered Rats. Data were presented as mean ± SD (n=6). CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic, CON = Control

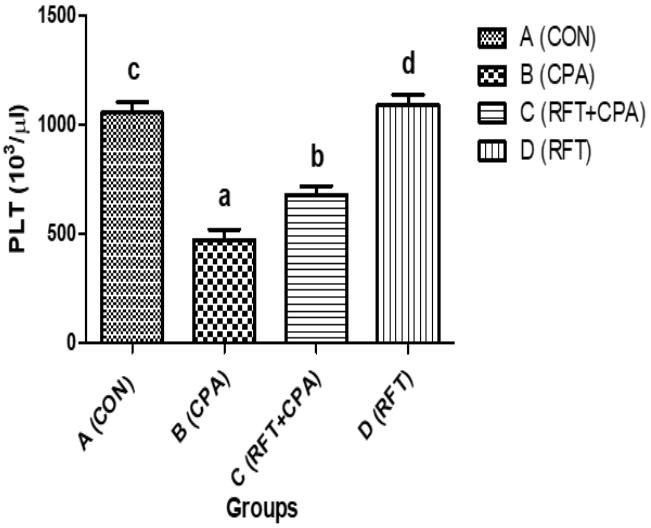

Figure 5. Influence of RFT on Platelet Count (PLT) in CPA-administered Rats. Data were presented as mean ± SD (n=6). CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic; CON = Control

Table 1 presents the influence of RFT on percentage (body temperature) change followed by CPA-administration in rats. The body temperature of animals that received only CPA injection (Group B) increased significantly ($p<0.05$) relative to normal control (Group A). The observed increase was recorded 24 h post-CPA administration and the value corresponds to a 5.1 % increase in body temperature. However, the animals (RFT+CPA; Group C) pre-treated orally for 7 days with RFT at 0.029 mL/kg prior to CPA-injection showed significant ($p < 0.05$) decreases by 2.5 % relative to animals that received CPA alone (Group B). While no significant difference ($p>0.05$) existed between animals that received only RFT (Group D) and the control (Group A).

Table 1. Influence of RFT on Body Temperature in CPA-administered Rats

Group	Initial body temp. (^{0}C)	Final body temp. (^{0}C)	Percentage change (%)
Control	36.2±0.38	36.3±0.38	↑0.3±0.03^a
CPA	36.5±0.40	38.5±0.42	↑5.1±0.18^c
RFT+CPA	36.4±0.42	37.4±0.40	↑2.6±0.15^b
RFT	36.1±0.35	36.2±0.45	↑0.2±0.02^a

Data were presented as mean ± SD (n=6). Results are significant at $p < 0.05$ with different superscripts down the column. CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic

Administration of CPA in rats altered the blood antioxidant balance, as monitored by the changes in the concentration of serum SOD, CAT and MDA (Table 2). Significant reduction in the concentration of SOD (11.87±0.34 U/mg protein) and CAT (1.31±0.05 µmol/min/mg protein) were observed in CPA control (group B) relative to normal control (25.04±0.46 U/mg protein and 3.82±0.08 µmol/min/mg protein respectively). However, animals (RFT+CPA; group C) pre-treated orally for 7 days with RFT at 0.029 mL/kg prior to CPA-administration significantly ($p<0.05$) increased the level of SOD to 16.42±0.38 U/mg protein, and CAT to 1.96±0.03 µmol/min/mg protein, relative to CPA control (group B). The observed increases correspond to 28 % and 33 % respectively. On the other hand, the MDA concentration increased significantly ($p<0.05$) to 3.35±0.07 nmol/mg protein, in the animals that received CPA alone (group B) relative to the normal control group (0.75±0.05 nmol/mg protein). However, MDA concentration significantly ($p<0.05$) reduced to 2.54±0.05 nmol/mg protein (approximately 32 %) in animals (RFT+CPA; Group C) pre-treated via oral administration for 7 consecutive days with RFT at 0.029 mL/kg prior to CPA-injection. Interestingly, oral administration of RFT for 7 consecutive days in rats improved the antioxidant system by 0.89 to 1.0-fold relative to normal control.

Table 2. Influence of RFT on Antioxidant Parameters in CPA-administered Rats

Group	Malondialdehyde (nmol/mg protein)	Superoxide dismutase (U/mg protein)	Catalase (µmol/min/mg protein)
Control	0.75±0.05^c	25.04±0.46^c	3.82±0.08^c
CPA	3.35±0.07^a	11.87±0.34^a	1.31±0.05^a
RFT+CPA	2.54±0.05^b	16.42±0.38^b	1.96±0.03^b
RFT	0.80±0.06^c	24.88±0.42^c	3.78±0.06^c

Data were presented as mean ± SD (n=6). Results are significant at $p < 0.05$ with different superscripts down the column. CPA = Cyclophosphamide; RFT = Ranferon-12 Tonic

DISCUSSION

There is unequivocal evidence that most anticancer agents often lead to several unwanted toxic side effects including haematotoxicity (Ferguson and Pearson, 1996; Testart-Paillet *et al.,* 2007). This particular side effect corresponds to haematopoietic dysfunction, which may be life-threatening to the patient undergoing CPA chemotherapy (Fan *et al.,* 2018; Iqubal *et al.,* 2020). To prevent such complications and optimize treatment, there is a need for an adjuvant therapy that can rebuild haematopoietic system. Ranferon-12 tonic (RFT) and other similar tonics contain haematinic factors capable of stimulating haematopoiesis (Haiden *et al.,* 2006; Remacha *et al.,* 2015). In this present work, RFT has shown an effect in mitigating cyclophosphamide-induced haematotoxicity in albino Wistar rats.

Haematotoxicity is one among several factors responsible for limiting the prognosis of neoplasms (Kameo *et al.,* 2021). One of the most reported mechanisms of CPA-induced haematotoxicity is the inhibitory effect of CPA or its xenobiotic metabolite (acrolein) on DNA replication causing oxidative damage and inhibiting the haematopoietic function of bone marrow cells (e.g. haematopoietic stem cells and haematopoietic progenitor cells) from producing peripheral blood cells (Liu *et al.,* 2014). Hence, measuring these peripheral blood cells count, directly mirrors the haematopoietic function (Tian *et al.,* 2021). In line with previous studies (El-Naggar *et al.,* 2015; Iqubal *et al.,* 2020; Kameo *et al.,* 2021), CPA administration at 200 mg/kg i.p. (24 h) from the study, led to a significant decrease in RBC, Hb, PCV, WBC and PLT levels; an indication of haematological abnormalities, due to decrease in peripheral blood cells. However, these reductions were mitigated to an extent, when pre-treated with RFT at 0.029 mL/kg for 7 consecutive days prior to CPA administration, suggesting that RFT possesses potential stimulants capable of enhancing haematopoiesis (Haiden *et al.,* 2006; Remacha *et al.,* 2015). This is because RFT consists of Fe, Vit B12 and folic acid; which seem to play a critical regulatory role in blood cells leading to haematopoiesis (Koury and Ponka, 2004; Mitra *et al.,* 2022). Although the mechanism remains to be explained, they are probably related to those promoting haematopoietic stem cell proliferation (Bills *et al.,* 1992; Liu *et al.,* 2015).

Previous reports have shown that balanced haematopoietic factors play a critical role in maintaining an effective haematopoietic system (Iqubal *et al.,* 2020). Under normal physiological conditions, haematopoietic factors regulate haematopoietic stem cells activity during haematopoiesis (Wang *et al.,* 2011). CPA or its xenobiotic metabolite (acrolein) causes alteration in the levels of these haematopoietic factors especially haematopoietic growth factors by producing excess pyrogenic cytokines and in turn leads to fever (Kameo *et al.,* 2021). Fever or pyrexia manifests as an increase in body temperature above the absolute temperature baseline regulated by the body's thermoregulatory center in the hypothalamus (Blatties, 2000; Pizzo, 1999). This finding clearly showed that CPA administration (at 200 mg/kg/day) causes fever, as evidenced by a 5.1 % increase in the body temperature of CPA treated group. Interestingly, the effect was reduced by 2.5 %, in the group pre-treated with RFT at 0.029 mL/kg for 7 consecutive days; suggesting that RFT might have modulated

haematopoietic growth factors, thereby suppressing the release of inflammatory pyrogenic cytokines (Kameo *et al.,* 2021).

Several studies have shown that CPA or its metabolite (acrolein) can bind to reduced glutathione and inhibits its antioxidant activity by producing more free radicals and causing lipid peroxidation, which is responsible for oxidative stress and cellular damage (Iqubal *et al.,* 2020; Kehrer and Biswal, 2000). In line with previous reports (Iqubal *et al.,* 2020; Patra *et al.,* 2012), this study has shown that a single administration of CPA at 200 mg/kg might increase the level of MDA content (an indicator of lipid peroxidation) and decrease the members of antioxidant defense enzyme (SOD and CAT levels). These suggest that CPA possesses powerful oxidative activity, which might have triggered the production of ROS and consequentially leads to tissue damage (Kehrer and Biswal, 2000). Since RFA does not have antioxidant capacity, alleviation of these effects by RFT might also be attributed to its ability to modulate haematopoietic factors and subsequently enhances haematopoiesis (Liu *et al.,* 2015).

CONCLUSION

The current finding revealed that RFT and other similar tonics could mitigate haematotoxicity in CPA-treated rats by suppressing inflammation/oxidative stress via modulation of haematopoietic factors, and in turn, promote haematopoietic stem cells as well as haematopoiesis. Thus, this study portrays RFT as a potential adjuvant in CPA-induced haematotoxicity.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest

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