

COMPARING THERAPEUTIC AND PROPHYLACTIC MODERATION OF PERTURBATION OF ERYTHROCYTIC AND PLATELET FUNCTIONS IN ULTRAVIOLET IRRADIATED RABBITS FED 2% TURMERIC SUPPLEMENT

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ABSTRACT: *Recent moderation of lympho-leukocytic responses of acutely ultraviolet (UV) irradiated pre-pubertal rabbits (PR) by 2% organic turmeric (Curcuma longa) supplement raises the possibility of wider protective implications for other blood components. This study thus investigated UV effects on erythrocytic/hematologic indices (EHI) and platelet (PLT) functions in PR and compares acute and chronic effects of T thereon. The study was for 85 days (d) in three (3) phased periods: 40d pre-irradiation, 5d irradiation and 40d post-irradiation in 60 acclimatized unsexed PR randomly assigned to 4 groups of 12 PR each as follows: Group 1, Control was fed un-supplemented diet and forage (Tridax procumbens) – basal diet (BD) for the entire study period without any treatment. Group 2 – T group (T+T+T) was fed BD supplemented with 2% pulverized crude T (BDS) during periods 1, 2 and 3, without irradiation. Group 3 – radiation (R) group (- +R+ -) was fed BD at periods 1, 2 and 3 and irradiated. Group 4 – therapeutic (H) group (- + R+T) was treated as in group 3 and fed BDS at period 3 only. Group 5 – prophylactic (P) group (T+TR+ -) was fed BDS during periods 1 and 2 only and irradiated. Feed and water were available ad libitum. Blood was collected on 86d from 0900h. Data were analyzed by ANOVA. UV significantly ($p < 0.05$) suppressed RBC count, hemoglobin concentration and hematocrit as well as platelet function. BDS normalized these variables towards control values. H application of T significantly ($p < 0.05$) elevated the variables to control values. A P application of T very significantly ($p < 0.05$) elevated values of these variables relative to acutely treated PR. These results demonstrate that T whether applied acutely or chronically moderated, UV induced perturbation of EHI and PLT functions of these PR.*

KEYWORDS: Turmeric, Therapy-Prophylaxis, Erythrocytic-Platelet-function, Ultraviolet radiation, Rabbits

INTRODUCTION

Mammalian blood and its constituent cell types have evolved and adapted to the performance of its vital metabolic and transport functions as well as protection of the body system and hence a successful environmental survival of the mammals. Lymphocytes are the blood effector cells for immune responses to antigens and foreign invading organisms. They defend the body against both infectious diseases and foreign materials. The immune system, according to Robberts (1995), involves a variety of white blood cells that work in concert to rid the body of the presence of foreign pathogens. The erythrocytes or red blood cells (RBCs) are the simplest mammalian blood cell. In the human body, they are formed nucleated in the bone marrow. They normally lose their nuclei prior to entering the circulation, and other organelles thereafter. By so doing, the RBC acquires its high surface to volume ratio in a functional adaptation necessary for its sole physiological function of gas transfer. Platelets like the RBCs are the other non-nucleated cells of the mammalian blood. Their hemostatic functions include

maintaining integrity of blood against loss and vascular injury/tear-mending. The human blood and its cellular components constitute a primary line of intracellular buffer/defense system. By their strategic ubiquity, these cells are highly sensitive and susceptible to the damaging effect of high energy rays such as UV.

Changing environment now impacts body systems negatively. These effects are paradoxically borne out of direct negative impact of the body system (i.e. humans) upon the environment itself. Increased solar UV radiation at environmental level, due to depletion of the stratospheric ozone layer (Mayer, 1992) and the accompanying negative impact have brought blood and its physiology to fore front of environmental scientific investigations. This depletion is global in nature. Its effect is more dramatic in tropical and subtropical regions. Thus, it calls for urgent serious research oriented interventions. It is responsible for potential acute effects and health hazards. For example, exposure to UV suppresses resistance against both systemic and non-skin-associated local infections. As well, UV exposure causes a variety of molecular changes which leads to immune suppression, down modulation of pre-existing cell-surface receptor, and a consequent suppression of some important functions of circulating blood borne phagocytic cells (Goettsch et al., 1996; Halliday et al., 2011).

We have recently demonstrated that organic turmeric (T) at a 2% supplementation level potently suppressed UV induced moderation of the WBC and absolute lymphocytic counts in pre-pubertal rabbits (PR). Furthermore, the demonstrated level of T prophylaxis appears to be time accentuated (Togun et al., 2014; Okwusidi et al., 2015). The imports of studies such as these are obvious. Potentially deleterious sub-dermal UV irradiation which generally permeates the skin surface albeit subtly may be responsible for various health hazards (Matsumura and Ananthaswamy, 2004, Bardak et al., 2000; Kitazawa and Iwasaki, 1999; Savoure et al., 1996; Bos et al., 1987). Thus, the demonstrated potent therapeutic/ (protective) prophylaxis of T supplementation of leuko-lymphocytic indices, a primary intracellular line of blood based defense becomes of basic and paramount complementation to the current understanding of animal organ-systems protection generally by traditional natural herbs such as T (Kapakos et al., 2012).

These results additionally suggest wider protective implications for T application in developmental and functional resilience of other components of the blood tissue in general. To this end, we have investigated the effect of UV irradiation on other blood parameters. This study evaluates a therapeutic T regimen on the erythrocytic and platelet functions in UV irradiated PR. In addition, the study also compares these responses to those of prophylactic setting as well. In other words, this study compares the acute (therapeutic) and chronic (prophylactic) effects of T supplementation in UV irradiated PR model.

MATERIALS AND METHODS

Experimental Site: The experiment was carried out at the Rabbitry unit of the Teaching and Research Farm, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.

Housing: The rabbitry with the cages were cleaned and disinfected. Cleaned and disinfected earthen feeders and drinkers were placed in each hutch before the rabbits were introduced into the hutches.

Design of Ultraviolet Radiation Chamber:

The UV box was designed as indicated by Togun et al. (2014) in such a way that the activities taking place within the chamber could be focally sampled through one of the sides of the box fitted with a transparent glass. All the other sides, including the entrance door side, were made of wooden planks, and covered with asbestos sheets. To prevent heat loss, the whole chamber, except the glass view side, was covered with a black polythene sheet as shown in Figure 1.



Figure 1: Ultraviolet Radiation Chamber - Showing rabbits being exposed

Dimension of the Ultraviolet radiation box is 1.07m by 0.6m by 1.08m.

The dosage of ultraviolet radiation received by each rabbit was calculated, using the formula by Podgorsak (2005), with reference to the body weight of the rabbits thus:

$$Dose = \frac{2PA \tan^{-1}(L)}{MLd (2d)}$$

P = Power rating of the UV tube

A = Cross Sectional Area of the animal

M = Mass/Weight of the animal

d = distance between the UV tube and the animal

t = period of exposure

L = length of tube

Processing of Turmeric: Organic turmeric rhizome was purchased from a certified organic farm at Odogbolu, Ogun State, Nigeria. The rhizomes were washed clean of sand and parboiled. They were sliced thinly and air-dried before being pulverized. The freshly pulverized unextracted material was further sieved through a cheese cloth to produce a uniform sized powder. This was added to the concentrate as test ingredient at 2% w/w inclusion rate.

Animal Handling and Experimental Protocol: The experimental protocol is essentially as described by Togun et al. (2014) and modified by Okwusidi et al. (2015). Sixty unsexed PR were obtained from a reputable local rabbitry. They were weight-balanced into 5 groups of 12 rabbits each and fed concentrate feed and daily generous supply of wilted *Tridax procumbens* plants (forage) as basal diet (BD). Table 1 summarizes proximate analysis of the minimum content of the concentrate feed.

The animals were acclimatized in standard individual hutches for 2 weeks before the commencement of the experiment. Following acclimatization, the rabbits were randomly allocated to four different feeding regimens and fed BD with or without 2% turmeric supplementation before, during and/or after irradiation as follows: Group 1, served as Control and was fed un-supplemented diet and forage (*Tridax procumbens*) – BD for the entire study period without any treatment. Group 2 – T group (T+T+T) was fed BD supplemented with 2% pulverized crude T (BDS) during periods 1, 2 and 3, without irradiation. Group 3 – Radiation (R) group (- +R+ -) was fed BD at periods 1, 2 and 3 and irradiated. Group 4 – therapeutic (H) group (- +R+T) was treated as in group 3 and fed BDS at period 3 only. Group 5 – prophylactic (P) group (T+TR+ -) was fed BDS during periods 1 and 2 only and irradiated. Feed and water were available *ad libitum*. Blood was collected on 86d from 0900h. The details of experimental design, protocol and treatment regimens are shown in Table 2.

Duration of Study: The experiment lasted for eighty five (85) days (d) in three phased periods of 40d (pre-irradiation), 5d (irradiation x 20 min^{-d}) and 40d (post-irradiation).

Experimental Design, Data Handling and Statistical Analysis: The experimental design and treatment regimen are summarized in Table 2. The experimental design was completely randomized block design. All values of measured variables are reported as mean \pm standard error of the mean (SEM). Values of measured variables were further normalized to control value and expressed as % of control value. Data were analyzed by Analysis of Variance (ANOVA) with graphic post-hoc test of significance. A $p < 0.05$ was considered statistically significant (Daniel, 1983; Godfrey, 1985).

RESULTS

Table 3 summarizes the values of measured erythrocytic indices in the control and PR fed T supplemented diet with or without UV irradiation. Compared to control, there was no significant difference in RBC count and hemoglobin (Hb) concentration of rabbits which were fed organic T-supplemented diet throughout the study period (T+T+T). In contrast, UV irradiation significantly ($p<0.05$) suppressed RBC count and diminished the Hb concentration in the irradiated rabbits, which were not fed the T supplemented diet either before or after irradiation (- + R + -), compared to the control or other treatment groups. Turmeric supplementation generally normalized these two parameters towards the control value.

Therapeutic (H) application of T supplementation (- +R+T) significantly elevated the RBC count and Hb concentration back towards control value compared to irradiated rabbits (Table 3). A pre-irradiative (chronic) prophylactic (P) T supplementation similarly and very significantly ($p<0.05$) elevated these variables. Compared to H effect, a P application of T significantly ($p<0.05$) countered more effectively the UV induced depression of these variables in the experimental animals. A similar trend was observed in the hematocrit (HCT) of the rabbits. Whereas UV irradiation significantly ($p<0.05$) reduced the HCT in the irradiated animals, T supplementation reverted the HCT values back to the control level especially in chronic prophylactic application.

Summarized also in Table 3 are the platelet (PLT) counts of the control and pre-pubertal rabbits fed T supplemented diet and with or without UV irradiation. The PLT count was significantly ($p<0.05$) higher in the rabbits that were fed T-supplemented diet throughout the study period (T+T+T) compared with the control group. On the other hand, UV irradiation significantly ($p<0.05$) suppressed PLT count in irradiated rabbits vis-à-vis the control and other groups. A P feeding of the T supplemented diet (T+TR+-) very significantly ($p<0.05$) increased PLT count relative to the control or other groups (Table 3).

Values of measured variables in the study were also normalized to the control value and expressed as % of control as depicted in table 4. Obvious from table 4, the PLT count as % of control values in these rabbits are H 212: P 264 compared to 219 and 71, values in the T+T+T and - + R + - (UV irradiated) groups respectively (Table 4).

Table 1: Proximate Analysis of the Concentrate Feed

Energy	2610.07MECa/kg
Crude Protein	18.4%
Crude Fiber	4.6
Ether Extract	4.6
Methionine	0.4
Lysine	0.8
Calcium	1.0
Phosphorus	0.2

Table 2: Experimental Design and Treatment Regimen

S/N	GROUP ⁿ	TREATMENT PHASES		
		Turmeric (T) (40 days)	Radiation (R) (5 days)	Turmeric (T) (40 days)
1.	CONTROL	-	-	-
2.	T + T + T	+	-	+
3.	- + R + -	-	+	-
4.	- + R + T	-	+	+
5.	T + TR + - (P)	+	+	-

n, number of animals per group = 12; + = plus; - = minus; H = Therapeutic; P = Prophylactic.

Table 3: Values of Measured Erythrocytic Indices and Platelet Count in Control and Pre-Pubertal Rabbits fed 2% Turmeric Supplemented Diet

S/N	GROUP ⁿ	PARAMETERS			
		RBC (10 ¹³ /l)	[Hb]g/dl	HCT (%)	PLT COUNT
1.	CONTROL	6.42±0.5 [†]	10.5±0.7	40.8±5	121.0±12
2.	T + T + T	6.00±0.5 ^{ns,*,§}	11.0±1.2 ^{ns,*,§}	38.7±4 ^{ns,*,§}	265.0±96 ^{*,**,b,c}
3.	- + R + -	5.10±1.4 ^{*,a,§}	7.4±1.0 ^{*,a,§}	33.3±6 ^{*,a,§}	86.0±26 ^{*,a,§,#}
4.	- + R + T (H)	5.90±0.4 ^{ns,γ,**,§}	9.60±0.6 ^{ns,γ,**,§}	39.8±5 ^{ns,γ,**,b}	256±92 ^{*,γ,**,§}
5.	T + TR + - (P)	6.60±0.5 ^{ns,a,**,#}	11.6±1.2 ^{ns,γ,**,#}	41.5±6 ^{ns,a,**,#}	319.0±110 ^{*,a,**,#}

n, number of animals = 12; [†]mean±SEM; **p*<0.05 vs control; ^a*p*<0.05 vs. T; ^{**}*p*<0.05 vs R; [§]*p*<0.05 vs P; [#]*p*<0.05 vs H; not significant (*ns*) vs Control; ^γ*ns* vs T; ^b*ns* vs P; ^c*ns* vs H; ^d*ns* vs R. T, Turmeric; RBC, Red Blood Cell; Hb, Hemoglobin; HCT, Hematocrit; PLT, Platelet. R, UV irradiation; P, Prophylactic; H, Therapeutic

Table 4: Values of Measured Erythrocytic Indices and Platelet Count as % of Control Value of Pre-Pubertal Rabbits fed a 2% Turmeric Supplemented Diet

S/N	GROUP ⁿ	% OF CONTROL			
		RBC	[Hb]	HCT	PLT COUNT
1.	CONTROL	100±10 [†]	100±10	100±10	100±10
2.	T + T + T	94±8 ^{ns,**, b, c}	105±8 ^{ns,**,§,#}	95±9 ^{ns,**,§,c}	219±20 ^{*,**,§,#}
3.	- + R + -	79±6 ^{*,a,§,#}	70.5±7 ^{*,a,§,#}	82±8 ^{*,a,§,#}	71±7 ^{*,a,§,#}
4.	+ - R + T (H)	92±8 ^{ns,γ,**,c}	91.4±2 ^{ns,a,**,b}	98±9 ^{ns,γ,**,b}	212±20 ^{*,γ,**,§}
5.	T + TR + - (P)	103±9 ^{*,a,**,#}	110.5±8 ^{ns,a,**,#}	102±9 ^{ns,γ,**,c}	264±25 ^{*,a,**,#}

n, number of animals = 12; [†]mean±SEM; **p*<0.05 vs control; ^a*p*<0.05 vs. T; ^{**}*p*<0.05 vs R; [§]*p*<0.05 vs P; [#]*p*<0.05 vs H; not significant (*ns*) vs Control; ^γ*ns* vs T; ^b*ns* vs P; ^c*ns* vs H; ^d*ns* vs R. T, Turmeric; RBC, Red Blood Cell; Hb, Hemoglobin; HCT, Hematocrit; PLT, Platelet. R, UV irradiation; P, Prophylactic; H, Therapeutic

DISCUSSION

This study has specifically evaluated the effect of UV irradiation on the erythrocytic indices and PLT functions in UV irradiated PR. It also compared the acute (therapeutic) and chronic (prophylactic) effects of organic T supplementation in these animals.

The results of this study suggest that acute exposure to UV rays seriously impacted the circulating RBC. UV rays reduced both the RBC count and its Hb concentration, and by extension the HCT of the rabbits in this study. These results are essentially similar to our previously observed reductive impact of UV on circulating levels of other component cells of the blood, leucocytes and lymphocytes (Togun et al., 2014; Okwusidi et al., 2015). Thus, in general, UV radiation strongly impacts negatively all blood cells. In contrast, T potently moderated the UV induced suppression of RBC count, reduction in Hb concentration, as well as a diminution of the HCT values whether consumed acutely or chronically.

The findings of this study especially demonstrated that a chronic prophylactic application of T supplementation alleviated the extent of UV induced suppression of the level of circulating RBC, reduction in Hb concentration and the accompanying diminution of packed cell volume or HCT. These observations are in tandem with evidence in literature and our previous findings which conclusively demonstrated a potent prophylactic effect of T in UV irradiated PR (Okwusidi et al, 2015). T and its main principle, curcumin have been implicated in disease remedy, antioxidant and anti-inflammatory properties including the exhibition of various biological effects such as anti-humoral, anti-ischemic and anti-hepatotoxic activities (Eigner and Scholz, 1999; Motolini et al., 2000; Singh et al., 2004). It is reputed to exert beneficial effects in multiple pathological conditions as well as possessing anti-inflammatory and antioxidant properties (Singh et al., 2004). In combination with other plant products, curcumin has been reputed to purify the blood and assist in menstrual and abdominal problems (Eigner and Scholz, 1999). Accordingly, in this study the animals fed a T supplemented diet prophylactically, showed greater resilience to the devastating effect of UV irradiation on RBC and Hb over their irradiated counterparts fed un-supplemented diet fed, buttressing those beneficial effects of T supplementation. Furthermore, this chronic (prophylactic) effect of T application appeared more effective than the acute short term therapeutic use.

Finally, exposure of the rabbits to UV rays in this study diminished markedly, the PLT count in the exposed rabbits relative to the unexposed animals. This finding is consistent with the findings of Zucker and Masiello (1984) and Van Marwijk Kooy et al. (1993) who noted similar platelet aggregation upon exposure to UV. Mechanistically, such PLT aggregation has been attributed to a possible oxygen radical induced activation of protein kinase C by UV-B (Van Marwijk Kooy et al., 1993). Our findings are further buttressed by several other reported cases of UV effects on PLT. In blood banking, various UV forms (A,B,C) have been used for preservative irradiation of PLT concentrate against microbial contamination during storage with varying degrees of success (Murphy et al., 2006; Johan et al., 2002). The limitation of such UV usage has been the damaging effect of UV on DNA and RNA, and in most cases the direct activation of PLT with consequent reduction in PLT quality, quantity, and coagulation complications (Zucker and Masiello, 1984; Terpstra et al., 2008; Benneth, 2008; Dorival et al., 2012). Thus, our observed decrease in PLT count is consistent with the theory of increased PLT aggregation.

In conclusion, the results of this present study have explicitly demonstrated that organic T at a 2% supplementation level potently moderated the UV induced perturbation of the erythrocytic

indices as well as the PLT functions of pre-pubertal rabbits whether applied acutely (therapeutically) or over a longer (chronic) prophylaxis. A more blinded study may help eliminate a possible placebo effect in the observed acute, therapeutic use or effect of T. In any case, the overwhelming prophylactic efficacy justifies the age long wide spread global multi-utility of organic T.

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