Prophylactic efficacy of turmeric (*Curcuma longa*) supplementation on the peripheral leukocytic response of pre-pubertal rabbits acutely irradiated with ultraviolet rays

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Recent experimental data had demonstrated potential pro-phylactic and therapeutic effects of a 2% Turmeric (*Curcuma longa*) diet regimen in peripheral leucocytic response (PLR) of pre-pubertal rabbits (R) acutely irradiated with ultra violet rays (UV). Thus, this study is a further elucidation of prophylactic efficacy of Turmeric (T) in UV irradiated R. The study was for 85 days (d) in three (3) phased periods: 40d pre-irradiation, 5d irradiation and 40d post-irradiation in 60 acclimatized pre-pubertal, unsexed R randomly assigned to 5 groups of 12 R each as follows: Group 1, served as control and 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 group (- +R+ -) was fed BD at periods 1, 2 and 3 and irradiated. Group 4 – neat prophylactic-1 (PP1) group (T+TR+ -) was fed BDS during periods 1 and 2 only and irradiated. Group 5 – the prophylactic-2 (PP2) group (T+TR+T) was treated as in group 4 but continued on BDS post-irradiation. Feed and water were available ad libitum. Blood was collected on 86d from 0900h. Data were analyzed by ANOVA. UV significantly (p<0.05) moderated PLR of BD fed R. Generally, BDS normalized PLR towards normal. Prophylactic application of BDS significantly (p<0.05) elevated PLR. Extended feeding of BDS to neat prophylactic group further into period 3 significantly (p<0.05) augmented PLR in these R. These results suggest that continued post-irradiative T-prophylaxis very potently ameliorated UV induced suppression of PLR in pre-pubertal rabbits.

**Key words:** Turmeric, Prophylaxis, Leukocytic-response, Ultraviolet-radiation, Rabbits.

INTRODUCTION

A recent study from our laboratory (Togun et al., 2014) evaluated the antioxidant/anti-inflammatory impact of organic turmeric (*Curcuma longa*) at enhancing developmental resilience in stress induced-ultra violet (UV) irradiated pre-pubertal rabbits, indexed by their peripheral leukocytic responses. The result from the study demonstrated that organic turmeric (T) supplementation significantly ameliorated the potentially deleterious UV-
induced suppression of WBC and absolute lymphocytic count in these rabbits. Leukocytes or white blood cells are cells of the immune system. They defend the body against both infectious diseases and foreign materials (Roberts, 1995). These cells are found throughout the body including the blood and lymphatic system. They constitute the first line of intracellular defense system. The number of leukocytes in the blood is often an indicator of disease. There is normally approximately 1% of total blood volume in a healthy adult (Albers, 2005). By their strategic ubiquity, normal lymphocytes are highly sensitive and susceptible to the damaging effect of UV. It has been reported that some of the UV rays from the sun or artificial light sources penetrate the epidermis and reach the dermis to affect the peripheral leucocytes, down modulating pre-existing cell-surface receptors (Bos et al., 1987; Matsumura and Ananthaswamy, 2004).

Ultraviolet (UV) radiation, an important part of the solar energy results in acute effects such as sunburn, photosensitivity reactions and immunosuppression known in humans. At high doses, UV has been implicated in the generation of oxidative stress with significant functional changes in various physiological systems in animals (Bardak et al., 2000; Kitazawa and Iwasaki, 1999; Savoure et al., 1996).

Currently, traditional herbs seem to enjoy more acceptance relative to prescription drugs in some cultures, resulting in considerable public and scientific interest in the use of plant derived products (phytochemicals) as prophylactic and therapeutic agents against a wide range of diseases (Kapakos et al., 2012). In addition, a number of dietary antioxidants e.g. flavonoids (a group of polyphenol compounds) exist beyond the phytochemicals. Such antioxidants are widely found in plants as glucosylated derivatives within the leaves, flowers, fruits, seeds, spices, medicinal plants and beverages. They are known to exhibit various biological effects such as anti-humoral, anti-ischemic, anti-hepatotoxic and anti-inflammatory activities (Shu, 1998).

Turmeric, with curcumin as the main active ingredient, is a tropical plant and a mandatory condiment in every Indian kitchen. It is extensively used as spice and food preservative as well as household remedy for diseases (Eigner and Scholz, 1999). Curcumin has been documented to exert beneficial effects in multiple pathological conditions as well as possessing anti-inflammatory and anti-oxidant properties (Singh et al., 2004).

The recent demonstration of ameliorative (prophylactic/therapeutic) effects of Tumeric (Togun et al., 2014) raises further this potential global use of Tumeric in multiple pathological conditions, as well as other interesting observations. Specifically, in the study (Togun et al., 2014), whereas a therapeutic application of Tumeric ameliorated irradiative effect of UV by moving the values of measured variables toward the control value, a prophylactic application significantly elevated these variables to and beyond the control values.

Thus, this study was specifically undertaken to further elucidate the prophylactic efficacy of Tumeric supplementation on UV irradiation in the pre-pubertal rabbit model.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Rabbitry unit of the Teaching and Research Farm, Ladoke 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, fixed 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.

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:

\[
Dose = \frac{3PAt \times 10^{-2}}{M \times d^2} (\text{J})
\]

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

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rhizomes were washed clean of sand and parboiled. They were sliced thinly and air-dried before being pulverized. The pulverized 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

Sixty unsexed pre-pubertal rabbits were obtained from a reputable local rabbitry. They were weight-balanced into 5 groups of 12 rabbits each and fed concentrate feed and a daily generous supply of wilted *T. 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 groups were randomly allocated to five 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 (*T. 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 group (- +R+ -) was fed BD at periods 1, 2 and 3 and irradiated. Group 4 – neat prophylactic-1 (PP1) group (T+TR+ -) was fed BDS during periods 1 and 2 only and irradiated. Group 5 – the prophylactic-2 (PP2) group (T+TR+T) was treated as in group 4 but continued on BDS post-irradiation. Feed and water were available *ad libitum*. Blood was collected on 86d from 0900h. The details of experimental design,
Table 1. Proximate Analysis of the Concentrate Feed.

<table>
<thead>
<tr>
<th>Energy</th>
<th>2610.07MECa/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>18.35%</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>4.63</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>4.63</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.43</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.76</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.9952</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.2131</td>
</tr>
</tbody>
</table>

Table 2. Experimental Design and Treatment Regimen.

<table>
<thead>
<tr>
<th>S/N</th>
<th>GROUP</th>
<th>TREATMENT PHASES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Turmeric (T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(40 days)</td>
</tr>
<tr>
<td>1.</td>
<td>CONTROL</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>T + T + T</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>- + R -</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>T + TR + - (PP1)</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>T + TR + T (PP2)</td>
<td>+</td>
</tr>
</tbody>
</table>

n, number of animals per group = 12; + = plus; - = minus, PP = Prophylactic 1, 2

Table 3. Values of Measured Variables in Control and Pre-Pubertal Rabbits fed a 2% Turmeric Supplemented Diet.

<table>
<thead>
<tr>
<th>S/N</th>
<th>GROUP</th>
<th>PARAMETERS</th>
<th>ABS LYM COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WBC (10^3)</td>
<td>LYM (%)</td>
</tr>
<tr>
<td>1.</td>
<td>CONTROL</td>
<td>5.30±0.60†</td>
<td>68.90±6.0</td>
</tr>
<tr>
<td>2.</td>
<td>T + T + T</td>
<td>5.03±0.50ns,*,§,#</td>
<td>78.60±4.0 ns</td>
</tr>
<tr>
<td>3.</td>
<td>- + R -</td>
<td>3.40±0.05a,§,#</td>
<td>70.20±4.0 ns</td>
</tr>
<tr>
<td>4.</td>
<td>T + TR + - (PP1)</td>
<td>6.67±0.60a,*,&quot;,§ #</td>
<td>77.50±5.0 ns</td>
</tr>
<tr>
<td>5.</td>
<td>T + TR + T (PP2)</td>
<td>10.10±1.00a,&quot;,§</td>
<td>72.80±3.0 ns</td>
</tr>
</tbody>
</table>

n, number of animals =12; †mean±SEM; *p<0.05 vs control; ‡p<0.05 vs T; ′p<0.05 vs R; §p<0.05 vs PP1; a,§p<0.05 vs PP2; not significant (ns) vs Control; ns vs T; ns vs PP1. T, Turmeric; R, UV irradiation; LYM, Lymphocyte; ABS, Absolute lym count; PP, Prophylactic 1, 2.

Duration of Study

The experiment lasted for eighty five (85) days (d) in three phased periods of 40d (pre-irradiation), 5d (irradiation) 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 ± standard error (SEM) of the mean (Table 3). Values of measured variables were normalized to control value and expressed as % of control value (Table 4). 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 depicts values of measured variables in the...
control and pre-pubertal rabbits fed 2% turmeric (T) – supplemented diet with or without UV irradiation. Compared to Control, there was no significant difference in leukocytic (WBC) count of rabbits (T+T+T) which were fed organic Turmeric supplemented diet throughout the study period. In contrast, UV irradiation significantly (p<0.05) suppressed leukocytic as well as absolute lymphocytic counts 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. Generally, Turmeric supplementation normalized leukocytic counts towards control value. A pre-irradiative (prophylactic, PP) application of T-supplementation (T+TR+-) significantly (p<0.05) elevated WBC count to control level (Table 3).

From the data, a continuation of T-supplementation of the prophylactically fed rabbits (T+TR+-) into period 3 (T+TR+T), significantly (p<0.05) augmented WBC recovery in these rabbits with the control or the neat prophylactic (PP) group (T+TR+-). An almost doubling of WBC value was noticed in the PP2 (T+TR+T) rabbits, compared to control or other groups. WBC values in these groups were 10.40, 6.67, and 5.30 at period 3, period 2 prophylaxes; control respectively (Table 3).

As shown in Table 4, a projection of T-supplementation (i.e. feeding of T-supplemented diet) onto phase 3, very significantly (p<0.05) improved the WBC recovery of PP2 rabbits. The WBC recovery as % of control value in these rabbits was 196, compared to the 125 value of the neat (PP) prophylactic (T+TR+-) group. The homologous values for the remaining groups were comparatively lesser than the prophylactic groups (Table 4).

**DISCUSSION**

This study specifically investigated an aspect of the prophylactic amelioration of potentially deleterious UV-induced suppression of leukocytic responses in pre-pubertal rabbit model. In this present study, Turmeric Supplementation was continued into the phase 3 of the study. This was essentially to study the recovery of the irradiated rabbits WBC and the effect of continued T-supplementation especially on the previously prophylactically fed rabbits, in other words extended prophylaxis (PP2).

The results from this current study are essentially consistent with and uphold our previous findings (Togun et al., 2014). Acute exposure to UV rays seriously impacted the circulating peripheral leukocytes, reducing both the leukocyte and absolute lymphocyte counts of the rabbits in this study. These findings are quite in agreement with published data. It has been reported that normal lymphocytes are highly sensitive to the damaging effect of UV radiation such as suppressing the immune system (Kripke, 1986) and consequent cell death (Matsumura and Ananthaswamy, 2004). Accordingly, our current results indicate that extended prophylactic Turmeric supplementation very potently moderated the UV-induced suppression of WBC and absolute lymphocytic counts in these pre-pubertal rabbits.

The results of this study have also convincingly demonstrated Turmeric associated prophylactic efficacy. These observations are in tandem with published literature evidence. Turmeric 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; Motelini et al., 2000; Singh et al., 2004). Indeed, similar Turmeric protective effects of host organ-systems as observed in this study have also been variously recorded in literature (Naovarat et al., 2011; Kapakos et al., 2012; Preeti et al., 2012; Gasem et al., 2013). Furthermore, Turmeric has been reputed to exert beneficial effects in multiple pathological conditions (Singh et al., 2004). In our study, animals fed a Turmeric supplemented diet during periods 1 and 2 only and subsequently irradiated exhibited greater resistance to the debilitating effects of UV irradiation over the

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**Table 4. Values of Measured Variables as % of Control value of Pre-Pubertal Rabbits fed a 2% Turmeric Supplemented Diet.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>GROUP</th>
<th>% OF CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CONTROL</td>
<td>100±00</td>
</tr>
<tr>
<td>2.</td>
<td>T + T + T</td>
<td>95±1.8&lt;sub&gt;a&lt;/sub&gt;γ,§,#</td>
</tr>
<tr>
<td>3.</td>
<td>- + R + -</td>
<td>64±1.17&lt;sub&gt;a&lt;/sub&gt;,§,γ</td>
</tr>
<tr>
<td>4.</td>
<td>T + TR + - (PP)</td>
<td>125±1.08&lt;sub&gt;a&lt;/sub&gt;,§,γ</td>
</tr>
<tr>
<td>5.</td>
<td>T + TR + T (PP)</td>
<td>196±1.72&lt;sub&gt;a&lt;/sub&gt;,§,γ</td>
</tr>
</tbody>
</table>

<sup>n</sup> number of animals =12; <sup>mean±SEM</sup>; <sup>p<0.05 vs Control</sup>; <sup>p<0.05 vs T</sup>; <sup>p<0.05 vs R</sup>; <sup><em>ns</em></sup> vs <em>a</em>; <sup>§</sup> vs <em>b</em>; not significant (<em>ns</em>) vs Control; <sup>ns</sup> vs <em>T</em>; <sup>γ</sup> vs PP; <sup>§</sup> vs PP; not significant (<em>ns</em>) vs Control; <sup>ns</sup> vs T; <sup>γ</sup> vs PP; <em>ns</em> vs <em>T</em>; <sup>§</sup> vs PP; <em>ns</em> vs PP.
irradiated, non-supplemented diet fed counterparts buttressing those beneficial effects of Tumeric supplementation. Chattopadhyay et al., (2004) reported that both turmeric and curcumin are well tolerated at high doses without any toxic effect. This is quite in tune with the observation in this study. The demonstrated Tumeric prophylaxis continued on till the conclusion of the study in those animals given extended Tumeric supplemented diet.

Lastly, the import of this study lies in the basic fact of the deleteriousness of sub-dermal UV irradiation which generally permeates the skin surface albeit subtly (Matsumura and Ananthaswamy, 2004; Bardak et al., 2000; Kitazawa and Iwasaki, 1999; Savoure et al., 1996; Bos et al., 1987). Thus, the currently demonstrated potent (protective) prophylaxis of Tumeric supplementation on leuko-lymphocytic indices, a primary intracellular line of defense becomes of basic and paramount complementation to the current understanding of animal organ-systems protection generally by T. The superior prophylactic efficacy of Tumeric supplement observed in this study vis-à-vis the therapeutic effectiveness (Togun et al., 2014) may explain the basis of the age long wide spread global utility of Tumeric as spice, condiment and in naturopathy. This Tumeric prophylactic efficacy could prove to be better acceptable relative to therapy, and further expand the uses of Tumeric.

All in all, the results of this study have conclusively demonstrated a potent prophylactic effect of Tumeric supplementation against UV-induced suppression of WBC and absolute lymphocytic counts in pre-pubertal rabbits. Furthermore, this Tumeric prophylaxis appears to be time accentuated. Finally, whether or not the prophylaxis is dose based is currently being investigated.

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REFERENCES


