European Journal of Biology and Medical Science Research
Vol.3, No.6, pp.48-56, December 2015
Published by European Centre for Research Training and Development UK (www.eajournals.org)

IMPLICATION OF RENAL BASED MECHANISM FOR ANTI-ULTRAVIOLET RAY RESPONSE TO ORGANIC TURMERIC SUPPLEMENT IN RABBITS

Okwusidi J.I.

Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

ABSTRACT: *Recent investigations failed to establish hepatic system as primary mechanistic locus for anti-UV effects of organic turmeric (T) in rabbits. Current effort, sought possible implication of renal mechanisms. Study was for 85days (d) in three phased periods: 40d pre-irradiation, 5d irradiation and 40d post-irradiation in 48 acclimatized rabbits randomly assigned to 4 groups of 12 each, fed unsupplemented diet and forage (Tridax procumbens) – basal diet (BD) and BD supplemented with 2% pulverized crude T. Feed and water were available ad libitum. Blood was collected on 86d from 0900h for measurement of renal parameters. Plasma (p) concentrations ($[\ ]_p$) of electrolytes were determined by flame photometry; urea, creatinine (CR) and HCO_3^- by standard titrimetric/colorimetric methods. Data were analyzed by ANOVA. $[Na^+]_p$ and $[HCO_3^-]_p$ in Control and T groups were statistically similar ($p>0.05$). UV moderated $[Na^+]_p$, $[HCO_3^-]_p$ and $[Urea]_p$ at 70% of control value (%C) and elevated $[CR]_p$ - %C 140, 100, 100: R, P, T groups respectively ($p<0.05$). T improved $[K^+]_p$ and revised suppressed $[Urea]_p$ at %C 70, 80, 90: R, P, T groups respectively ($p<0.05$). These results strongly implicate renal system as a primary mechanistic locus.*

KEYWORDS: Turmeric, Prophylaxis, Renal-mechanism, Ultraviolet-radiation, Rabbits.

INTRODUCTION

Recently observed increases of solar UV radiation at environmental level have been attributed to depletion of the stratospheric ozone layer (Mayer, 1992). Such solar effect impacts the human body systems negatively. This potentially deleterious sub-dermal UV irradiation which generally permeates the skin surface albeit subtly, may be responsible for various health hazards (Savoure et al., 1996; Kitazawa and Iwasaki, 1999; Bardak, 2000; Matsumura and Ananthaswamy, 2004; Bos, 1987). It has become a new primary "vector" of aberrant mammalian physiology, which is now subject to a host of recent researches worldwide.

Many plants/natural products such as turmeric, ginger, garlic etc, perhaps because of their pharmaco-complaisance, are currently employed in naturopathy to counter some of these potentially deleterious effects of this changing environment. Recent experimental data from this laboratory have demonstrated potential prophylactic and therapeutic effects of a 2% organic turmeric (curcuma longa) diet regimen in peripheral leucocytic response of pre-pubertal rabbits (PR) acutely irradiated with ultra violet rays (UV). The results from the studies strongly upheld the ameliorative effects of T whether applied prophylactically or therapeutically (Okwusidi, 2015a). The mechanism(s) by which T ameliorates these effects remain largely obscure. In recent observations, the response of various blood components exhibited addictive rather than synergistic pattern of response to T supplementation in UV irradiated PR (Okwusidi et al., 2015, Okwusidi, 2015a,b). In concert, these observations suggest a possible complimentary organ-based mechanism of action rather than supplementary mechanism and/or possibly both. Thus, the possible mechanistic role of the hepatic system in

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

organ-based anti-UV tissue response to organic T by UV-irradiated PR has more recently been evaluated (Okwusidi, 2015c).

The results from this latter study demonstrated a significant modulation of the activities of hepatic function marker enzymes. However, a key function of the liver, protein synthesis indexed as measured total protein (TP) or albumin (ALB) concentrations was not appreciably affected. Thus, in that instance, the study seems to have failed to categorically establish mechanistically, the hepatic system as a primary locus for the ameliorative actions of organic T supplement against UV effect (Okwusidi, 2015c).

The hepatic and renal systems play elementary roles in body nutrients-electrolyte fluxes, fluid dynamics and other conjoint functions in the body. Specifically, the renal system of the mammals serve essential regulatory and metabolic roles such as regulation of electrolytes, maintenance of acid-base balance and regulation of blood pressure via the maintenance of salt and water balance. In addition, the renal system acts as a natural filter of the highly UV vulnerable blood (Togun et al., 2015; Okwusidi, 2015a, b, c). It removes in the form of urine, wastes such as urea, essentially produced in the liver. As well, the renal system rids the body of creatinine (CR), an amino acid found as a metabolic waste product of CR, an important energy storage molecule in the mammalian muscle. These waste products need to be excreted by the kidney. A marked increase of these parameters in the blood suggests functional damage to the renal system (Panda, 1999).

These unique functions of the renal system outlined above form the premises upon which the renal or kidney function tests are based. The tests evaluate how well the kidneys work, whether they are failing to performs their functions by measuring the waste products left behind in the blood. Some of these tests are specific, while other are not. For example, manifest urea blood level can be influenced by many factors including dehydration, anti-diuretic drugs and diet. These variables make the urea test non-specific. On the other hand, CR test is more specific to the kidney since kidney damage is the only significant factor that will increase serum CR level. Nonetheless, increased levels of the both parameters correlate with increases in protein catabolism and consequent decreases in total protein concentration; and thus, impairment of kidney function (Cheesbrough, 1998).

Lastly, the various renal mechanisms of handling electrolyte and organic substances are pretty well known and established. They help define renal functions generally. Thus, specific impact of various experimental interventions can to a certain extent be predicted. Due to versatility of reported T applications including medicinal purposes, it is logical and expedient to systematically research critically its ability to prevent adverse effects of UV radiation on renal functions.

This present study thus sought a possible implication of renal mechanism in the anti-UV ray tissue response of prophylactic T supplementation observed in PR acutely irradiated with UV rays.

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.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

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.

Figure 1: Ultraviolet Radiation Chamber showing exposure of rabbits

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:

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

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

where:

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 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: Forty-eight unsexed pre-pubertal rabbits (PR) were obtained from a reputable local rabbitry. They were weight-balanced into 4 groups of 12 PR each and fed concentrate feed and a 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.

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

Following acclimatization, the rabbits were randomly allocated to four different feeding regimens and fed un-supplemented diet and forage (*Tridax procumbens*) – basal diet (BD) with or without 2% turmeric supplementation before or during irradiation as follows: Group 1, served as control and was fed 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 – Prophylaxis 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 by marginal venopuncture, for measurement of renal parameters. Plasma concentrations of electrolytes were determined by flame photometry; urea, creatinine (CR) and HCO_3 by standard titrimetric/colorimetric methods.

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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 details of experimental design, protocol and treatment regimens are shown in Table 2.

Table 2: Experimental Design and Treatment Regimen

GROUP [n]	TREATMENT PHASES		
	Turmeric (40 days)	**Radiation** (5 days)	**Turmeric** (40 days)
CONTROL	-	-	-
T + T + T	+	-	+
- + R + -	-	+	-
T + TR + -	+	+	-

*n, number of animals per group = 12; T, Turmeric; R, UV irradiation; P, Prophylaxis (*T + TR + -), *+ = plus; - = minus*

The experimental design was completely randomized block design. All values of measured variables are reported as mean ± standard error (SEM) of the mean. Values of measured variables were 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

The values of measured renal parameters in the control and pre-pubertal rabbits fed a T-supplemented diet with or without UV irradiation are displayed in table 3.

Table 3: Renal Parameters in Pre-Pubertal Rabbits fed a 2% Organic Turmeric Supplement

PARAMETER	GROUP [n]			
	CONTROL	T + T + T	- + R + -	T + TR + -
[K^+] (mEq/L)	$6.2\pm0.3^{\dagger}$	$6.9\pm0.6^{ns,**,b}$	$5.1\pm0.8^{*,a,\#}$	$6.0\pm0.4^{ns,\gamma,**}$
[Na^+] (mEq/L)	153 ± 2.0	$154\pm4.0^{ns,**,b}$	$144\pm6.0^{*,a,\#}$	$150\pm3.0^{ns,\gamma,**}$
[HCO_3] (mEq/L)	22.3 ± 2.0	$23.1\pm1.0^{ns,**,b}$	$18.33\pm1.6^{*,a,\#}$	$24.4\pm2.0^{ns,\gamma,**}$
UREA (mg/dL)	8.0 ± 1.3	$7.0\pm0.7^{*,**,\#}$	$5.6\pm0.5^{*,a,b}$	$6.0\pm0.5^{*,a,d}$
CREATININE (mg/dL)	52.4 ± 8	$59.5\pm11^{*,**,\gamma}$	$73.5\pm9^{*,a,\#}$	$60.3\pm12^{*,**,\gamma}$

*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; not significant (ns) vs Control; $^{\gamma}$ns vs T; ^{b}ns vs P; ^{d}ns vs R. T, Turmeric; R, UV irradiation; P, Prophylaxis (*T + TR + -), *+ = plus; - = minus.*

The plasma Na^+ concentration in the Control and T supplemented groups were essentially the same. Neat T supplementation had no significant ($p>0.05$) effect on plasma Na^+ concentration. As well, the bicarbonate concentrations in these rabbits were not statistically different from the control ($p>0.05$).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

In contrast to the effect of T applications, UV irradiation significantly ($p<0.05$) moderated the concentrations of measured plasma electrolytes - Na^+, K^+ and HCO_3^-. By contrast, neat T supplementation significantly ($p<0.05$) improved the plasma K^+ concentration stance in treated animals, when compared with the control and other groups. UV irradiation significantly reduced plasma urea concentration in irradiated rabbits, when compared to Control and T treated groups ($p<0.05$). The urea concentration in these animals was 70% of the control value (Table 4).

Table 4: Renal Parameters as % of Control Values in Pre-Pubertal Rabbits fed a 2% Organic Turmeric Supplement

GROUP [n]	PARAMETER (% OF CONTROL)				
	[K^+] (mEq/L)	**[Na^+]** (mEq/L)	**[HCO_3]** (mEq/L)	**UREA** (mg/dL)	**CREATINE** (mg/dL)
CONTROL	$100\pm10^{\dagger}$	$100\pm10^{\dagger}$	$100\pm10^{\dagger}$	$100\pm10^{\dagger}$	$100\pm10^{\dagger}$
T + T + T	$100\pm2.0^{ns,**,b}$	$100\pm2.0^{ns,**,\#}$	$100\pm0.5^{ns,**,b}$	$90\pm05^{ns,**,b}$	$100\pm14^{ns,**,b}$
- + R + -	$80\pm3.0^{*,a,\#}$	$90\pm3.0^{*,a,\#}$	$80\pm0.8^{*,**,\#}$	$70\pm0.4^{*,a,\#}$	$140\pm11^{*,\gamma,a}$
T + TR + -	$100\pm1.3^{ns,\gamma,**}$	$100\pm1.5^{ns,\gamma,**}$	$100\pm1.0^{ns,\gamma,**}$	$80\pm0.4^{*,\gamma,*}$	$100\pm15^{ns,\gamma,**}$

*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; not significant (ns) vs Control; [γ]ns vs T; [b]ns vs P; [d]ns vs R. T, Turmeric; R, UV irradiation; P, Prophylaxis (*T + TR + -*), + = plus; - = minus.*

A neat T supplementation significantly ($p<0.05$) revised the UV suppressed plasma urea concentration in these rabbits when compared with Control or other experimental groups (Table 3). As compared to the control, the average urea concentration in these animals as % of control value was 90% (Table 4). Prophylactic application of T generally maintained electrolytes concentration around the control value. This is in contradistinction to the significant UV induced depletion of these electrolytes ($p<0.05$). Urea concentration was significantly ($p<0.05$) elevated towards the Control and T treated group over the period of prophylaxis when compared to the UV group. The urea concentrations as a % of control value were 70, 80, 90: R, P, T groups respectively (Table 4).

Table 3 also depicts the mean plasma concentrations of CR in the Control and T supplemented rabbits with or without UV irradiation. Compared to the Control and other groups, UV irradiated rabbits had significantly ($p<0.05$) higher CR value. The CR concentration in this UV groups as % of control value was 140% compared to 100 (T) and 100 (P) groups. A neat T supplementation improved CR concentration relative to the control PR. Similar effects were noticed in prophylactic application of T. The UV-elevated CR value was significantly ($p<0.05$) moderated by T prophylaxis vis-à-vis the Control.

There were no significantly ($p>0.05$) noticeable differences in CR concentrations of the neat- and prophylactically fed PR (Table 3). The CR concentrations as % of control value in these groups were 100 each respectively (Table 4). Therapeutic effects of T supplementation in evaluated PR were essentially similar to observed prophylactic effects (data not shown).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

DISCUSSION

This study is part of a continuous effort to explain the basis of the ameliorative T effect on UV-pathophysiology. UV-rays as a result of changing environment continue to impose constraints of human physiology. For example, without excluding other sub-dermal effects, acute effects of UV irradiation include sunburn, photosensitivity reactions and immunosuppression. The kidney has essentially two main homeostatic functions: To excrete metabolic wastes from the body and regulate the water and ionic contents of the blood. Thus, the kidney as a homeostatic organ (Bernard, 2009) is a logical site of lesion in these instances.

The results of this study have explicitly demonstrated that the renal system is a prime mechanistic locus for T ameliorative anti-UV effects in PR.

Evident from the results of this study, acute exposure to UV radiation seriously impacted negatively all the plasma electrolytes, urea as well as the CR concentrations; thus demonstrating the deleteriousness of acute UV irradiation. The diminished electrolytes level suggests possible impact on fluid dynamics – fluid shift in the case of Na^+ ions and loss of vital electrolytes, K^+ and HCO_3^-, in these animals.

Urea is a nitrogenous compound which is produce in the liver from the oxidation of amino acids or ammonia and is transported to the kidney for excretion. It acts as a carrier for waste nitrogen and also plays a role in the countercurrent exchange system for the nephron, which allows for re-absorption of water and critical ions from excreted urine. Indeed, urea allows the kidney to conserve body water by creating hyperosmotic urine (i.e. more ions (concentrated) in it). Preventing water loss in this way is critical to attaining proper water balance, the maintenance of suitable blood pressure or suitable concentration of Na^+ ions in the blood/plasma. Thus, the moderation of serum/plasma urea concentration by acute UV irradiation relative to Control and other groups of this study further highlights the deleterious potential of UV irradiation.

The increased mean serum/plasma CR concentration in these irradiated PR confirms that UV irradiation is nephrotoxic and may be responsible for kidney damage at extremes. This finding is in perfect agreement with Panda (1999), that exposure to UV irradiation leads to elevated levels of CR suggestive of impaired renal functions. CR is more specific to the kidney since damage to the kidney is the only significant factor which increases plasma/serum CR concentration.

Finally, the findings of this study demonstrated that a T supplementation alleviated the extent of UV induced suppression. The observed normalization of the UV moderated plasma/serum electrolytes and urea concentrations, with the moderation of the UV elevated CR concentration by T supplementation is a pointer to the nephroprotective properties of T. These observation are in tandem with our previous findings (Okwusidi et al., 2015; Okwusidi 2015a,b,c) and evidence in literature that 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 Scholaz, 1999; Motelini et al., 2000). The results of this study also agree with the findings that upon exposure to UV irradiation, pretreatment with T resulted in preservation of histological integrity with a diminution of tubular damage and restoration of glomerular and Bowman's capsule functions. Normal levels of serum urea, uric acid and CR were also restored (Shoshes, 1998).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

CONCLUSION

In conclusion, the results of this study have explicitly demonstrated that the renal system is a primary mechanistic locus for the anti-UV ray responses of T supplementation observed in acutely UV irradiated PR.

Acknowledgement

Gratitude is extended to Mr. Tobi Adetunji for professional typesetting and ICT dexterity and Barr. CNN Okwusidi for language and proofing. This work was supported in part by grants-in-aid of research from Tecobic Nig Ltd and NigCan Packers Co. Ltd Gratis.

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ISSN 2053-406X(Print) ISSN 2053-4078(Online)