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ENHANCED TESTICULAR FUNCTIONAL INDICES IN 2% TURMERIC SUPPLEMENTED UV-IRRADIATED RABBITS

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ABSTRACT

Testicular function is modified by maturational gonadostatic control highly susceptible to negative physiologic niche-altering factors like UV-rays. We evaluated effects of UV irradiation and organic turmeric (T) on testicular functional indices in 40 rabbits. Study was for 85days (d) in three phased periods: 40d pre-irradiation, 5d irradiation and 40d post-irradiation in 40 acclimatized rabbits randomly assigned to 4 groups of 10 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. EDTA blood was collected on 86d from 0900h for estimation of indices of testicular function. Plasma (p) concentrations ($[\ ]_p$) of TE, LH, FSH and TCHOL were determined enzymatically. Data were analyzed by ANOVA. $[TE]_p$ and $[FSH]_p$ in T group was significantly higher than Control and other groups with % of Control (%C) values 170/120% : TE/FSH ($p<0.05$). $[LH]_p$ and $[TCHOL]_p$ of the group were similar to Control, %C values 100/90 : LH/TCHOL ($p>0.05$). UV significantly suppressed all measured plasma variables, %C values 24/80% : TE/FSH; 8/70% : LH/TCHOL ($p<0.05$). Prophylaxis mimicked neat T supplementation, %C values 120/120% : TE/FSH ($p<0.05$); 100/90% : LH/TCHOL ($p>0.05$). These results strongly suggest that UV severely impairs, while organic T amelioratively enhances testicular functional indices.

INTRODUCTION

The testicle is the male gonad of mammals. Like the ovaries to which they are homologous, the testes are components of the reproductive and endocrine systems. The ultimate functions of the testes in mammalian reproduction are distinctively dual in nature. Firstly, is the production of viable sperm cells in adequate proportion in both quantity and quality. To do this the testes employ enzymes and hormones. The seminiferous tubules contain the germ cells and perform the physical testicular sertoli (spermatogenic) function, as well as the exocrine function of releasing the produced spermatozoa into its lumen. These spermatozoa are subsequently washed into the epididymis by fluid secreted by the sertoli cells. In the second testicular (androgenic) function, the interstitial tissue performs the endocrine aspect of testicular function. It synthesizes and secretes androgens. Specifically, the leydig (or interstitial) cells synthesize testicular hormones, Testosterone (TE) being the primary sex hormone of the mammalian male gender. Age dependent modulating dynamics of these two essential functions occur by way of hormones of the hypothalamic-pituitary axis: gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH) and to an extent prolactin (Prl or 5-HT). Their interactions with target tissue and the hypothalamus together constitute the so called *gonadostatic control* and maturation which are akin to the mammalian reproductive physiology.

The mammalian sperm cells are aerobes. Like other oxygen breathing, aerobic cells, they are constantly challenged by the so called "oxygen paradox". On the one hand, oxygen is essential to life in these cells. Catabolism of oxygen yields reactive species (ROS) which at physiological concentration drives the necessary spermatogenic function [1-4]. On the other hand, these ROS can impair cell function and limit cell survival [1]. Due to the inefficiency of our endogenous defense systems as well as the existence of some negative physiological altering situations such as air pollutants, cigarette smoke and ultraviolet (UV) radiation, the human body generally reacts defensively by forming reactive hybrid of vitamins and biomolecules. As a result of

pollutants, ROS can be produced in excess, thus increasing the amount of dietary antioxidants load necessary to diminish the cumulative effect of oxidative damage over an individual's life span [5].

UV-irradiation as a result of environmental denudation caused by the depletion of the protective stratospheric ozone layer [6], has recently become a "new vector of aberrant mammalian physiology". This changing environment continues to impose constraints on human physiology. The physiological stress on the body that is caused by the accumulative damage done by free radicals, which are not adequately neutralized by anti-oxidants is known as oxidative stress (OS).

Recent evidence has incriminated defective spermatogenic function as the most causality of human male infertility [7]. A possible cause of this infertility which is of current research focus is the generation of ROS in the male reproductive system. The human semen is believed to contain different cell types including sperm cells at various stages of maturity, epithelial cells, leukocytes and others. The leukocytes, especially neutrophils and macrophages produce ROS. When produced in large amounts, ROS have potential toxic effect on sperm quality and function. Indeed, recent reports indicate that high levels of ROS are detected in the semen samples of 25 to 40% of infertile men [1].

Turmeric (T), with curcumin as its main active ingredient, is a tropical plant and a mandatory condiment in every Indian kitchen. It is extensively used as a spice and food preservative, as well as a household remedy for diseases [8, 9]. Curcumin is highly pleiotropic and interacts with numerous targets. It has been documented to exert beneficial effects in multiple pathological conditions as well as possessing anti-inflammatory and anti-oxidant properties [10]. Recent experimental data from our laboratory have demonstrated that organic T potently impacted the erythrocytic indices and platelet function in pre-pubertal rabbits (PR) in a time accentuated manner [11-13]. To a large extent leukocytic response constitutes the first line of intracellular defence in infectious process [14]. We have demonstrated that T modulated UV induced WBC response and absolute lymphocytic count [11, 12]. The mechanism(s) of T action in these instances appear to be acute in nature, possibly based at the renal level [15], with a suggestive hepatic synergism [16].

The effect of testicular function is not fully resolved. As well, there is paucity of basic correlative research data on this subject. This study thus specifically investigated the effects of UV and T supplement on testicular function in a PR 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 [11] 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 [17], with reference to the body weight of the rabbits thus:

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

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 rhizomes were 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 concentrate feed as test ingredient at 2% w/w inclusion rate.

Animal Handling and Experimental Protocol: Forty male PR were obtained from a reputable local rabbitry. They were weight-balanced into 4 groups of 10PR 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 aclimatization, the PR were randomly allocated to four different feeding regimens and fed BD with or without 2% turmeric supplementation before or during 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 group (- +R+ -) was fed BD at periods 1, 2 and 3 and irradiated. Group 4 – prophylactic (P) group (T+TR+ -) was fed BDS during periods 1 and 2 only and irradiated. Feed and water were available *ad libitum*. EDTA anticoagulated blood was collected on 86d from 0900h by marginal venopuncture, for measurement of indices of testicular function: Testosterone (TE), luteinizing hormone (LH), follicle stimulating hormone (FSH). Total plasma cholesterol concentration (TCHOL) was also determined.

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

Experimental Design, Data Handling and Statistical Analysis: The details of experimental design, protocol and treatment regimens are summarized in Table 2. The experimental design was completely randomized block design. All values of measured variables are reported as mean ± standard error of the mean (SEM). Values of measured variables were further normalized to control value and expressed as % of control value (%C). Data were analyzed by Analysis of Variance (ANOVA) with graphic post-hoc test of significance. A $p<0.05$ was considered statistically significant [18, 19].

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.	T + TR + -	+	+	-

n, number of animals per group = 10; T, Turmeric; R, UV irradiation; T + TR + -, Prophylaxis (P); + = plus; - = minus

RESULTS

The values of measured indices of testicular function: TE, FSH, LH and TCHOL of control and rabbits fed 2% supplement with or without radiation are depicted in table 3. Their corresponding %C values are summarized in table 4.

Table 3: Indices of Testicular Function and Plasma Total Cholesterol in Rabbits fed 2% Turmeric Supplement

GROUP [n]	PARAMETER			
	TE (pg/ml)	LH (µg/ml)	FSH (µg/ml)	TCHOL (µmol/l)
CONTROL	$1.50\pm0.20^{\dagger}$	0.70 ± 0.07	0.50 ± 0.04	1.30 ± 0.10
T + T + T	$2.60\pm0.30^{*,**,\#}$	$0.70\pm0.07^{ns,**,b}$	$0.60\pm0.06^{*,**,b}$	$1.20\pm0.10^{ns,**,b}$
- + R + -	$0.40\pm0.04^{*,a,\#}$	$0.60\pm0.06^{*,a,\#}$	$0.40\pm0.04^{*,a,\#}$	$0.90\pm0.10^{*,a,\#}$
T + TR + -	$1.80\pm0.20^{*,a,**}$	$0.70\pm0.06^{ns,\gamma,**}$	$0.60\pm0.05^{*,\gamma,**}$	$1.20\pm0.10^{ns,\gamma,**}$

*n, number of animals =10; †mean±SEM; *p<0.05 vs control; ap<0.05 vs. T; **p<0.05 vs R; #p<0.05 vs. P; not significant (ns) vs Control; γns vs T; bns vs P; dns vs R. TE, Testerone; LH, Luteinizing Hormone; FSH, Follicle Stimulating Hormone; TCHOL, Total Plasma Cholestrol. T, Turmeric; R, UV irradiation; T + TR + -, Prophylaxis (P); + = plus; - = minus.*

Table 4: Indices of Testicular Function and Plasma Total Cholesterol as % of Control Value in Rabbits fed 2% Turmeric Supplement

GROUP [n]	PARAMETER			
	TE (pg/ml)	LH (µg/ml)	FSH (µg/ml)	TCHOL (µmol/l)
CONTROL	$100\pm10^{\dagger}$	100 ± 10	100 ± 10	100 ± 10
T + T + T	$170\pm20^{*,**,\#}$	$100\pm10^{ns,**,b}$	$120\pm10^{ns,**,b}$	$90\pm8^{ns,**,b}$
- + R + -	$24\pm3^{*,a,\#}$	$8\pm1^{*,a,\#}$	$80\pm8^{*,a,\#}$	$70\pm7^{*,a,\#}$
T + TR + -	$120\pm12^{*,a,**}$	$100\pm10^{ns,\gamma,**}$	$120\pm10^{ns,\gamma,**}$	$90\pm8^{ns,\gamma,**}$

*n, number of animals =10; †mean±SEM; *p<0.05 vs control; ap<0.05 vs. T; **p<0.05 vs R; #p<0.05 vs. P; not significant (ns) vs Control; γns vs T; bns vs P; dns vs R. TE, Testerone; LH, Luteinizing Hormone; FSH, Follicle Stimulating Hormone; TCHOL, Total Plasma Cholestrol. T, Turmeric; R, UV irradiation; T + TR + -, Prophylaxis (P); + = plus; - = minus.*

Compared to Control and other experimental groups, the TE and FSH concentrations were very significantly ($p<0.05$) higher in the rabbits which were fed organic T supplement throughout the period (T+T+T). The concentration of LH and TCHOL in this group were not statistically different ($p>0.05$) from control values (Table 3). The %C values in this T+T+T group were 170/120% : TE/FSH and 100/90% : LH/TCHOL respectively (Table 4).

In contrast to the effect of T application, UV irradiation significantly ($p<0.05$) suppressed the concentrations of measured plasma indices of testicular function as well as TCHOL concentration, when compared to the Control or T supplemented rabbits. The %C values in these UV irradiated rabbits were 24/80% : TE/FSH and 8/70% : LH/TCHOL respectively. Prophylactic application of T (T+TR+-) more or less mimicked the neat T supplementation, with a significant ($p<0.05$) elevation of TE and FSH concentrations. Prophylaxis generally maintained the LH and TCHOL around the control values. The values of these latter variables were essentially statistically similar ($p>0.05$) to the Control (Table 3). The %C values were 120/120% : TE/FSH and 100/90% : LH/TCHOL respectively (Table 4).

DISCUSSION

Normal regulation of mammalian testicular function is fairly stratified with a classic gonadostatic control. It is characterized by a relative pre-pubertal high hypothalamic sensitivity to sex steroid, TE feedback control, through medial sensitivity of puberty to the adult level sensitivity. Pre-pubertally, for example in the humans, the carry-over of human chronic gonadotropin (HCG) induced TE production of the infantile testis persists to maintain the relatively high concentration of TE to which the hypothalamus is very sensitive. The hypothalamus thus quickly responds with decreased release of gonadotropins. This high hypothalamic sensitivity is responsible for the maintenance of low plasma concentrations of gonadotropins and androgens typical of the pre-pubertal period in the males. It forms the basis of our current use of a classic pre-pubertal herbivorous animal model in this case rabbits, to study the physiologic regulation of testicular function in omnivores, with a probable human relevance.

The result of this study have demonstrated that acute exposure to UV-rays potently moderated measured plasma indices of testicular function, TE, FSH and LH, as well as TCHOL. These findings are consistent with evidence in literature on the toxicity of UV-irradiation. The potentially deleterious sub-dermal UV irradiation which permeates the skin surface albeit subtly may be responsible for various health hazards even at cellular level [20, 21]. For example, when organisms are exposed to UV irradiation in the presence of oxygen and photosensitive component of cells such as gem cell line, ROS are formed. This in turn, results in oxidative stress [21].

The diminished TCHOL observed in this study, probably represent the UV augmented cholesterol catabolism and other cellular cholesterol dependent sinks such as hormone production and so on [22, 23]. The findings of this study especially demonstrated that prophylactic T supplementation alleviated the extent of UV induced suppression of the level of circulating FSH, LH and the accompanying diminution of TCHOL. These observations are in tandem with evidence in literature and our previous findings [12]. T and its main principle, curcumin have been implicated in a plethora of disease remedy, antioxidant and anti-inflammatory properties including the exhibition of various biological effects such as anti-humoral, anti-ischemic and anti-hepatotoxic activities [9, 10, 25].

The results of this study are equally not at variance with other published data. Similar to T induced elevation of TE observed in this study, Liao et al [25] had described increased TE concentration which they ascribed to curcumin inhibition of the activity of the enzyme, 5α-Retuctase activity. This enzyme catalyzes the conversion of TE to 5α – dihydrotestosterone (5α - DHT), a pre-requisite for the morphogenetic action of TE at tissue level. Such an inhibition probably spares TE and hence its increase at substrate level.

Finally, T is believed to possess anti-fertility effect [26] in mostly pubertal/cycling experimental animals [27] of perhaps lessened gonadostatic TE-hypothalamic sensitivity in contrasting comparison to pre-pubertally maturing experimental animals as used in the present study. Obviously therefore, TE-hypothalamic feedback potency vary between testicular maturation, a situation that will explain our observed elevation of FSH concentration and hence the augmented testicular (spermatogenic) function in T-supplemented animals of this study. This axiom is further buttressed by the non-significant effect of T supplementation on LH/TCHOL concentrations (a more or less androgenic function) which were essentially at control level.

CONCLUSION

In conclusion, the results of this study have explicitly demonstrated that UV-irradiation seriously impacts testicular function negatively. Organic T supplementation potently ameliorates this UV effect. These results are consistent with the thesis that in contradistinction to pubertal/adult anti-fertility role, T supplementation at 2% level promotes follicular stimulation and testicular spermatogenic function in UV irradiated pre-pubertal rabbits.

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