Contents lists available at www.innovativejournal.in

INNOVATIVE JOURNAL OF MEDICAL AND HEALTH SCIENCE

Journal homepage: http://innovativejournal.in/ijmhs/index.php/ijmhs

ISSN No. 2277-4939

Research Article

A DOSE RESPONSE STUDY: GRADATION OF PROTECTIVE EFFECT OF TURMERIC ON UV-INDUCED NEPHROTOXICITY IN RABBITS

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Ω To a fond memory of Prof. Togun V.A. who passed-on during this work. R.I.P. *Prof.*

ARTICLE INFO

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Keywords: Dose-response-study, Turmeric, Ultraviolet-radiation, Nephrotoxicity-indices, Rabbits.

DOI:http://dx.doi.org/10.15520/ijmhs.2016.vol6.iss1.103.

ABSTRACT

A dose-response study has been undertaken to clarify our proposed mechanistic axiom relating manifest UV-induced nephrotoxicity to protective effects of gradated turmeric (T) supplementation. Study was for 20days (d) at exposure rate of 50min/d for 5d in 50 acclimatized pre-pubertal, unsexed rabbits (PR) randomly assigned to 5 groups of 10PR each, fed concentrate feed and forage (*Tridax procumbens*) – basal diet (BD) and BD supplemented with 0% (Control), 2%, 4%, 6% and 12% pulverized crude T respectively. Feed and water were available *ad libitum*. Blood was collected 24h after the last exposure by marginal venopuncture up till 21d from 0900h for assessment of renal nephrotoxicity indexed as changes in plasma/serum concentrations of urea and creatinine (CR) determined by standard titrimetric/colorimetric methods. Data were analyzed by ANOVA. Dose dependent increases in secretion of both indices were observed. Highest amount of secreted urea occurred in rabbits fed 2% T followed by 4% T and were significantly ($p<0.05$) higher than control and 6% T rabbits. At 12% T amount of measured urea dropped very significantly ($p<0.05$) below control value with a reversal to negative value. Similar increasing trend occurred in CR secretion, exhibiting initial non-significant ($p>0.05$) splay at 2% T followed by continuous significant increases ($p<0.05$). Unlike urea, highest CR secretion occurred at 6% T and without a reversal to negative value. These results strongly demonstrated potent dose dependent nephroprotection by gradated T supplementation.

INTRODUCTION

Increased environmental solar UV radiation as a result of depletion of the stratospheric ozone layer [1] continues to impose constraints on human physiology. It impacts the human organ-systems negatively. For example in addition to other sub-dermal effects, the acute effects of UV irradiation include sunburn, photosensitivity reactions and immuno-suppression. The mammalian renal system is a primary homeostatic organ with two principal functions. It excretes metabolic wastes from the body and regulates ionic contents of the blood. In conjunction with the hepatic system, the kidney plays elementary role in the body nutrient-electrolyte fluxes, fluid dynamics and other conjoint functions in the body. Thus, the kidney as a homeostatic organ [2] is a logical site of lesion in UV pathology. Indeed, UV radiation seriously impacts negatively all aspects of renal function - the plasma electrolytes, urea as well as the creatinine (CR) concentrations; thus demonstrating the deleteriousness of acute UV irradiation [3].

Urea is a nitrogenous compound, produced 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 and osmotic stratification along nephron, which allows for re-absorption of water and critical ions from excreted urine. Urea allows the kidney to conserve body water by creating hyper-osmotic 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. As well, the renal system rids the body of CR, an amino acid found as a metabolic waste product of creatine, 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 [4]. CR is more specific to the kidney since damage to the kidney is the only significant factor which increases plasma/serum CR concentration. These basic axioms form the premise for the use of manifest abnormal plasma/serum levels of these compounds, urea and CR as indices of renal nephrotoxicity in this study.

We have demonstrated that 2% organic turmeric supplement in whatever form administered positively impacted anti-UV (patho)physiological response(s) of many organ-systems including blood cells of irradiated pre-pubertal rabbits (PR) model [5, 6, 7]. These responses are discrete as well as time accentuated. For example, a doubling in WBC recovery response, erythrocytic indices and platelet function was recorded in anti-UV response of irradiated rabbits in extended T prophylaxis [8]. The mechanism(s) of these anti-UV effects of T remain largely and relatively obscure. Our laboratory data however suggest that the mechanism(s) are acute in nature, based in the renal bed [3], and possibly in synergism with the hepatic system [9]. Whether or not additivity of responses to a specific dose application translate into a true dose-response effect as in these studies remains to be fully elucidated.

This study was thus construed to relate manifest UV-induced nephrotoxicity, indexed as modulation of plasma/serum urea and CR concentrations to protective effects of gradated T supplementation.

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 [10] 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 (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 [11], with reference to the body weight of the rabbits thus:

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

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

Animal Handling and Experimental Protocol: Fifty unsexed PR were obtained from a reputable local rabbitry. They were weight-balanced into 5 groups of 10 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, irradiated rabbits were randomly allocated to five different feeding treatment regimens and fed un-supplemented diet and forage (*Tridax procumbens*) – basal diet (BD) with or without gradated turmeric supplementation: Group 1, served as control and was fed BD for the entire study period (i.e. 0% BDS). Group 2 was fed BD supplemented with 2% pulverized crude T (BDS). Group 3 was fed 4% BDS. Group 4 was fed 6% BDS. Group 5 was fed 12% BDS. Irradiated rabbits were exposed to 50min/day of acute ultraviolet radiation for 10 days. Feed and water were available *ad libitum*. Blood was collected 24h after the last exposure up till 21d from 0900h by marginal venopuncture, for assessment of renal nephrotoxicity. Plasma concentrations of urea and creatinine (CR) were determined by standard titrimetric/colorimetric methods.

Duration of Study: The experiment lasted for twenty (20) days (d).

Experimental Design, Data Handling and Statistical Analysis: The experimental design and treatment regimen are as outlined in Table 2, in a completely randomized experimental design.

Table 2: Experimental Design and Treatment Regimen

S/N	GROUP [n]	TREATMENT	
		Radiation (5 days)	**Turmeric**(20 days)
1.	CONTROL	+	-
2.	2% T	+	+
3.	4% T	+	+
4.	6% T	+	+
5.	12% T	+	+

n, number of animals per group = 10; T, Turmeric; R, UV irradiation; + = plus; - = minus

All values of measured variables are reported as mean ± standard error of the mean (SEM). Values of measured variables were normalized to control value, followed by dose correction. Data were analyzed by Analysis of Variance (ANOVA) with appropriate post-hoc test of significance. A $p<0.05$ was considered statistically significant [12, 13].

RESULTS

The values of measured renal nephrotoxicity indices, urea and CR in the control and pre-pubertal rabbits fed a gradated T-supplemented diet are displayed in table 3.

Table 3: Nephrotoxicity Indices in rabbits fed gradated organic turmeric supplement

S/N	GROUP [n]	PARAMETER	
		UREA (mg/dL)	**CREATININE** (mg/dL)
1.	CONTROL	$5.0\pm0.5^{\dagger}$	59.2 ± 6
2.	2% T	$7.2\pm0.7^{*,\gamma,\beta,\#}$	59.6 ± 6^{ns}
3.	4% T	$6.3\pm0.6^{*,\S,\beta,\#}$	$63.6\pm6^{*,\beta,\#,a}$
4.	6% T	$5.1\pm0.5^{\S,\beta,\#,ns}$	$77.6\pm8^{*,\S,\gamma,\#}$
5.	12% T	$3.8\pm0.4^{*,\S,\gamma,\beta}$	$66.4\pm4^{*,\S,\gamma,\beta}$

*n, number of animals per group =10; †mean±SEM; *p<0.05 vs Control, §p<0.05 vs 2%, γp<0.05 vs 4%, βp<0.05 vs 6%, #p<0.05 vs 12%; not significant (ns) vs control; anS vs 2%*

Table 4 summarizes the values of dose corrected indices of nephrotoxicty in the control and rabbits fed gradated turmeric supplement. A dose dependent increase in the secretion (as adjudged by measured plasma/serum concentrations of both indices) was observed. The highest amount of secreted urea was observed in the rabbits fed 2% T, followed by 4%; and were significantly ($p<0.05$) higher than the control and 6% supplemented rabbits. In these latter rabbits, there was no significant difference in the urea concentrations in both the control and 6% T supplemented rabbit ($p>0.05$).

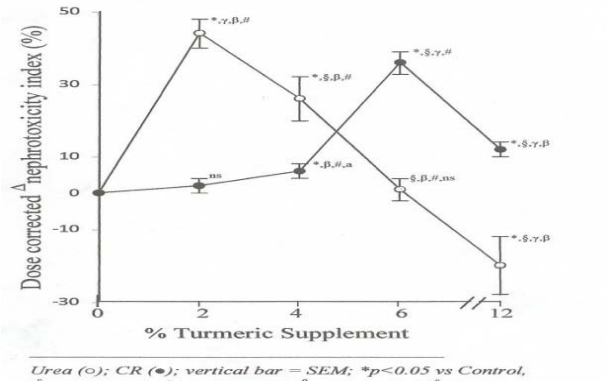
Table 4: Dose corrected indices of nephrotoxicity in rabbits fed gradated organic turmeric supplement

S/N	GROUP [n]	PARAMETER			
		UREA (%)		**CREATININE** (%)	
1.	CONTROL	$0\pm0^{\dagger}$	-	$0\pm0^{\dagger}$	-
2.	2% T	$44\pm4.0^{\dagger,*}$	↑↑↑	0.68 ± 0.7^{ns}	↑
3.	4% T	$26\pm3.0^{*}$	↑↑	$7.4\pm0.7^{*,\S,\S}$	↑↑
4.	6% T	$1.0\pm0.1^{*}$	↑	$31.0\pm2.0^{*,}$	↑↑↑
5.	12% T	$-20/\pm4^{*,\gamma,**}$	↓↓	$12.2\pm1.0^{*,ns}$	↑↑

*n, number of animals per group =10; †mean±SEM; *p<0.05 vs Control, §p<0.05 vs 2%, γp<0.05 vs 4%, βp<0.05 vs 6%, #p<0.05 vs 12%; not significant (ns) vs control; anS vs 2%. Arrow indicates direction and magnitude of modulation.*

At 12% T supplementation level, the amount of urea measured had dropped precipitously when compared with Control and other supplement levels (Table 4, arrows). The values of urea in this 12% T rabbits dropped very significantly ($p<0.05$) below the control value to the point of a negative value reversal/reflection as displayed in figure 2.

Figure 2: Effect of Gradated Turmeric supplementation on renal nephrotoxicity indices



*Urea (○); CR (●); vertical bar = SEM; *p<0.05 vs Control, §p<0.05 vs 2%, γp<0.05 vs 4%, βp<0.05 vs 6%, #p<0.05 vs 12%; not significant (ns) vs control; anS vs 2%.*

Similar increasing trend was observed in the secretion of CR. Compared to Control, CR concentration exhibited an initial modest but non-significant ($p>0.05$) increase at 2% supplementation level. This was followed by continuous and significant ($p<0.05$) increases in the concentration of secreted CR. In contrast to urea secretion, highest concentration of secreted CR occurred at 6% T supplementation. As depicted in Figure 2, the secretion of CR at 12% T was significantly ($p<0.05$) lower than that at 6% T supplementation, but still significantly ($p<0.05$) higher than control and other T supplemental levels. In further contradistinction, there was no negative value reversal/reflection in CR secretion as was the case in urea secretion/excretion (Figure 2).

DISCUSSION

Dose response relationships or studies generally serve important heuristic functions in physiologic/pharmacologic investigations. They either act as pointer to establishment of a mechanistic hypothesis for example, or as in this study buttress or clarify a proposed mechanistic axiom. Various published data have demonstrated dose response relationship in various experimental animal settings in trying to explain the plethora of biological activities associated with T supplementation. T through its main principle, curcumin is highly pleiotropic and interacts with numerous targets [14-16].

The results of this study have demonstrated a potent dose response relationship between gradated T supplementation and the extent of nephrotoxicity manifested in our studied rabbits. T supplementation at all levels impacted positively the secretion/excretion of both urea and creatinine (CR). The maximal secretion/excretion observed at 2% T in the case of urea and 6% T in CR. At 12% T, the value of secreted urea had reversed into negative value in contrast to the CR secretion. In other words, the kidney at 12% T was starting to retain (or reabsorb) urea, hence the precipitous decline in the amount of measurable urea. The 12% T, CR secretion was significantly lower than value at 6% T but still significantly higher than their control and other T supplemented counterparts.

These observations are quite in tune with basic renal physiological principles/mechanisms. Normally, CR is a nephrotoxic waste product of muscle metabolism. At the Bowman capsule, CR is freely filtered but not reabsorbed. Thus its secretion/excretion rate equals the rate at which it was filtered. This therefore allows secretion/excretion of essentially all the filtered CR. Urea on the other hand is very toxic to the body. Therefore, it is also normally freely filtered but it is more or less reabsorbed (albeit poorly). This also means that normally large quantities of the compound are excreted in the final urine. Indeed about 40% of filtered urea is known to be normally found in final urine because aided by anti-diuretic hormone, there is more re-absorption than secretion along the nephron. This pre-excretory recycling of urea helps to maintain the kidney/nephron countercurrent/rectifier mechanisms and thereby sustain osmotic stratification along the nephron and hence kidneys ability to create hyperosmotic (concentrated) urine. Thus, the results of T supplementation in this study have again demonstrated the acuteness of its nephroprotective potency by facilitating the secretion/excretion of nephrotoxic urea. The urea negative value reversal observed at 12% T probably

represent a physiologic homeostatic escape response to prevent the erosion of the osmotic stratification along the nephron, and a potential washout of the kidneys urine concentrating ability, all due to hyper-effectiveness of T action.

Finally, although detailed pharmacodynamics/ kinetics evaluations were not undertaken in this study, the 2% T and 6% T nephrotoxicity values observed in our rabbits represent values below the LD_{50} for frank renal nephrotoxicity in these animals. This stance augurs well with evidence in literature. Al-Noori et al [17] demonstrated that curcuma longa powder at 1% supplementation of their basal diet increased WBC in chickens. Our study similarly showed a wide range of dose tolerance of T without any apparent toxicity, similar to variously observed non-toxic high dose tolerances of T. Thus, a general consensus exists as to curcumin being tolerated and safe at high doses [18, 14-16). However, our study also revealed a potential untoward effect of higher doses of curcumin on renal osmotic stratification in our PR model as per urea excretion if not for renal homeostatic escape which intervened in this case to re-absorb urea, thus conserving the renal *status quo* ability to form concentrated urea.

CONCLUSION

In conclusion, the results of this study have demonstrated a very potent and effective dose response relationship in the ameliorative effect of organic turmeric on UV-irradiation in PR model. Physiological homeostatic mechanism(s) appear to intervene to prevent such high dose turmeric/curcumin over-effectiveness to erode normal functioning of the renal nephron of UV-irradiated PRs. Such basic physiological principles should always be put into consideration in quests for development of acceptable curcumin formulations as therapeutic agent.

Acknowledgement:

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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How to cite this article: Okwusidi J I, Togun V A,. A Dose Response Study: Gradation Of Protective Effect Of Turmeric On UV-Induced Nephrotoxicity In Rabbits. **Innovative Journal of Medical and Health Science**, [S.l.], v. 6, n. 1, feb. 2016. ISSN 2277-4939. Available at: <http://innovativejournal.in/ijmhs/index.php/ijmhs/article/view/103>. Date accessed: 15 Feb. 2016. doi:10.15520/ijmhs.2016.vol6.iss1.103.