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Tissue histology and antioxidant defense system in female rats exposed to sun rays.

Ayobola Abolape Iyanda[1], Chinedum Iheanacho Iheakanwa[2]

[1] Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.
[2] Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan. Nigeria

Email: lapeiyanda@yahoo.com, pastorchinedum@yahoo.com

Abstract – Although considerable distance separates the earth and the sun- the star at the center of the Solar System- yet its harmful effects after excessive exposure to ultraviolet rays of the sun have been identified as a cause of many pathological conditions. The role of lipid peroxidation in various disease states has also been highlighted. The aim of the study is to investigate whether 4 hourly exposure to sunlight is capable of inducing tissue damage that is oxidative stress-mediated. Fourteen female Wistar rats of 13 weeks of age were distributed into 2 groups. Sun-exposed rats were left in an open place without any form of sun-screen for protection against sunlight. Blood was collected from the animals by retro-orbital bleeding. The serum obtained was used for the estimation of activities of catalase, superoxide dismutase, glutathione S transferase, glutathione reductase and glutathione peroxidase, as well as levels of malondialdehyde (MDA), and reduced and oxidized glutathione. Sections of the brain, lung, heart, and ileum were processed and stained with haematoxylin and eosin (H&E). Statistical analysis was carried out using the Student's t test. $P \leq 0.05$ was considered significant. Results showed that there were significant increases in serum levels of MDA and GSSG as well as significant decreases for GSH, Gln-px and SOD, an indication of the involvement of oxidative stress in histologic changes recorded for some of these tissues. Histologic changes like mild congestion of the coronary vessel (heart) as well as mild pulmonary congestion (lung). The absence of histolgic changes in tissues like kidney and liver even when some of the oxidative stress markers were significantly different and suggestive of free radical generation, may be as a result of the fact that free- radical are highly reactive with very short half-lives, which means they combine almost immediately after their production in situ.
Keywords – Sun ray, Tissue histology, Antioxidant

1. Introduction

Lipid peroxidation is a process of oxidative deterioration of polyunsaturated fatty acids that leads to the formation of hydroperoxides, short-chain aldehydes, ketones, and other oxygenated compounds. This process has been linked with the development of various diseases that affect many tissues. Some of the diseases that have been clearly demonstrated to be linked with lipid peroxidation include atherosclerosis [1], diabetes [2], and cancer [3]. Lipid peroxidation has also been identified as one of the main contributing factors in aging [4]. Free radical-mediated lipid peroxidation has even been proposed to be critically associated with some other diseases like brain dysfunction, and cardiovascular disease; its role in the degenerative processes has also been highlighted. Enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) protect cell membranes from lipid peroxidation.

According to many sources, the Sun is the star at the center of the Solar System that is almost perfectly spherical and consists of hot plasma interwoven with magnetic fields. Its diameter of about 1,392,000 km, makes it about 109 times that of Earth, and its mass (about 2×1030 kilograms is 330,000 times that of Earth) accounts for about 99.86% of the total mass of the Solar System [5]- [8]. Although considerable distance separates the earth and the sun yet the beneficial effects of the sun is felt greatly on earth. For example, sunlight is responsible for photosynthesis as well as vitamins D precursor synthesis yet its harmful effects after excessive exposure to ultraviolet rays of the sun have also been reported. The aim of this study is to determine whether tissue damage (brain, lung, heart, ileum) occurs after 6 weeks of exposure to sunlight from 9.00 to 12.00 and is oxidative-stress mediated. This will be achieved through histologic examination of the brain, lung, heart and ileum as well as assessment of serum levels of oxidative markers e.g. SOD, CAT, Gln-px, GST, Gln-reduc, MDA, GSH, and GSSG.

2. Materials and Methods

2.1. Animals and Animal Care

The experimental animals used for the study consisted of fourteen female Wistar rats of 13 weeks of age. The study which was for a period of 6 weeks was carried out at the Experimental Animal Unit of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. Of the fourteen rats, seven constituted the sun-exposed group and the other seven rats served as control. Daily from 9:00 to 13:00 five times a week, sun-exposed rats were left in an open place without any form of sun-screen for protection against sunlight. The control rats were kept in cages at ambient temperature of 25°C and a 12 h light, 12 h dark cycle. All experimental animals were supplied feed and water without any form of restriction. Blood collection that was through retro-orbital

bleeding took place between 10:00 and 12:00. The blood was dispensed into anti-coagulant free bottle, and centrifuged at 3000 g for ten minutes. The serum obtained was stored at -20ºC until required for analysis. Animal treatment was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

2.2. Assessment of select serum anti-oxidants and MDA

Activities of catalase, superoxide dismutase and glutathione peroxidase and MDA were quantified by the methods of Sinha [9]; Kakkar et al. [10]; Rotruck et al. [11]; and Ohkawa et al. [12] respectively. The activity of glutathione S transferase was determined using the method of Habig et al. [13], but that of glutathione reductase activity was by method of Zhou & Freed [14]. Levels of reduced and oxidized glutathione were assessed by employing the methods of Prins and Loos [15] and Owen Joshua and Butterfield [16] respectively.

2.3. Histopathology

The animals were sacrificed and sections of the following tissues: brain, lung, heart, and ileum were cut and fixed in 10% neutral buffered formalin. The tissues were dehydrated in ascending concentration of ethanol, cleared in xylene and embedded in paraffin block. Sections of 5 µm were made using motorized rotary microtome. After which they were stained with haematoxylin and eosin (H&E), slides were then examined under compound light microscope and histopathological changes were assessed. The slides were viewed under the microscope at magnification of ×400.

2.4. Statistical analysis

Data obtained are expressed as mean ± SD (standard deviation). Level of significant difference between the two groups was determined using Student's t test. SPSS package version 15 was used for this purpose. $P \leq 0.05$ was considered significant.

3. Results

The results of the study are presented in Tables 1 and 2 as well as figures 1 and 2 below. In Table 1, serum levels of reduced glutathione, oxidized glutathione, reduced/oxidized glutathione ratio and malondialdehyde were significantly different in sun-exposed rats compared with control ($p<0.05$). Moreover in Table 2, catalase and glutathione peroxidase were significantly different in sun-exposed rats, whereas glutathione reductase, superoxide dismutase and glutathione S transferase were not significantly different ($p>0.05$). While all the tissues examined for the control rats showed no visible lesion as shown in Figure 1. Results presented in Table 2 for sun-exposed rats included heart (mild congestion of the coronary vessel); lung (mild pulmonary congestion); brain (no visible lesion); and ileum (no visible lesion).

Table 1: Serum levels of reduced glutathione, oxidized glutathione, reduced/oxidized glutathione ratio and malondialdehyde.

	GSH (mol/ml)	GSSG (mol/ml)	MDA (nmol/ml)	GSH/GSSG Ratio
Control	1.49±0.19	0.15±0.20	16.06±1.74	15.00±1.94
Sun-exposed	1.15±0.14*	0.21±0.09*	31.87±5.91*	5.48±0.65*

Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with control using Student's t test. Abbreviations: GSH-reduced glutathione; GSSG-oxidized glutathione; GSH/GSSG-reduced/oxidized glutathione ratio; MDA- malondialdehyde.

Table 2: Serum activities of glutathione reductase, catalase, glutathione peroxidase, superoxide dismutase and glutathione S transferase

	Gln reduc (U/mg protein)	CAT (µmol H_2O_2 consumed/(min·mg protein))	Gln-px (µmol GSH consumed/(min·mg protein)	GST (U/mg protein)	SOD (U/mg protein)
Control	60.00±6.05	4.67±0.49	18.96±4.04	0.75±0.59	11.74±1.98
Sun-exposed	59.86±7.71	3.40±0.51*	14.30±3.11*	0.79±0.73	9.50±.0.87

Results are expressed as mean ± standard deviation. Abbreviations: SOD-superoxide dismutase; CAT- catalase; Gln-Per- glutathione peroxidase; Gln-reduc - glutathione reductase; GST- glutathione S transferase. *p <0.05 is significant

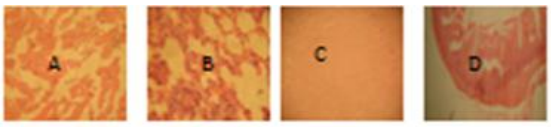

FIG 1: Below are the photomicrographs of different tissues of control rats: A- heart (no visible lesion); B- lung (no visible lesion); C- brain (no visible lesion); D- ileum (no visible lesion). Mag. X 400

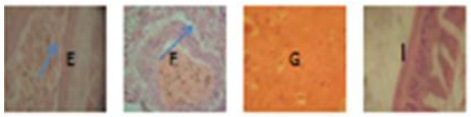

FIG 2: Below are the photomicrographs of different tissues of sun-exposed rats: E- heart (mild congestion of the coronary vessel); F- lung (mild pulmonary congestion); G- brain (no visible lesion); H- ileum (no visible lesion). Mag. X 400

4. Discussion

Foreign chemical substances that are harmful to the body exert their toxic effects through a number of mechanisms. Many of these agents are metabolically processed to yield the reactive (intermediate) metabolites. For example acetaminophen (APAP) is biotransformed through the cytochrome 450 pathway to produce *N*-acetyl- *p*-benzoquinone imine (NAPQI) that is highly reactive and damaging to both hepatic and renal cells. The sun emits

different kinds of rays that have been identified to affect lives on earth in a variety of ways. While agents like APAP, aflatoxin B1 and a number of others are known chemical compounds with proven highly toxic effects, ultraviolet B (UVB) is an example of physical agents with established carcinogenic and toxic properties.

In many cases of xenobiotic-induced tissue damage that is oxidative stress mediated, not only are there histopathological manifestations, in most cases serum indices of oxidative stress such as SOD, GSH, Gln-px, CAT, Gln-reduc, GST, etc always complement histology results. While the role of UVB on an organ like the skin is well proven, observations have also been made in the past that have linked excessive sun exposure with organs such as the eyes (cataract, photoconjunctivitis). The sun has also been identified to play a role in many skin diseases e.g. basal cell carcinoma and squamous cell carcinoma. The skin is the organ that is most highly susceptible to the effect of the sun, because of its close proximity with the sun rays. And sun exposure-induced skin pathological conditions have been known to be free radical mediated [17]. That the sun does this through oxidative stress-mediated processes can be deduced from the findings of Toyokuni et al. [18].

Toyokuni et al. [18] highlighted that exposure to sunlight is a major factor in the deterioration of skin function. Their study involved the use of thirty-six fixed human skin samples from sun-exposed and unexposed areas from young and old individuals to evaluate the localization of oxidative stress according to levels and distribution of oxidative stress marker 8-hydroxy-2'-deoxyguanosine in samples by immunohistochemistry. Results of their study showed that in the epidermis of young subjects, negligible amounts of 8-hydroxy-2'-deoxyguanosine were detected in unexposed areas, whereas nuclear 8-hydroxy-2'-deoxyguanosine was increased in the lower epidermis in sun-exposed areas.

Data from the study of Toyokuni et al. [18] as well as Nishigori et al. [19]; Wlaschek et al. [20] seem to suggest that there is evidence that UV-induced skin damage is mediated by reactive oxygen species (ROS), which target virtually all biomolecules. Changes in genomic DNA are closely linked with carcinogenesis. Proteins on the other hand are also important in the skin as structural components for the protection of the underlying cells [21]- [22]. Many of the abnormal skin manifestations resulting from excessive sun exposure e.g. basal cell carcinomas are likely to be products of such modifications. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is one of the main oxidatively modified DNA bases, that is induced by hydroxyl radical, singlet oxygen, photodynamic reaction or peroxynitrite, and is mutagenic when present in DNA replication [19], [23], [24]. Increase in the amount of epidermal 8-OHdG after single UV exposure at high doses [25] – [27] as well as after chronic repeated exposure at low doses [26]- [27] in mice have been reported.

While this study did not involve measurement of 8-OHdG, an indicator frequently used to evaluate oxidative stress; other indices like levels of MDA, GSH, GSSG, Gln-reduc, SOD, CAT and activities of antioxidant enzymes were evaluated. Significantly differences were recorded for many of these markers compared with control, which signifies the systemic oxidative-potential of sun rays. In addition, MDA an indicator of lipid peroxidation was also significantly increased. Lipid peroxidation takes place when hydroxyl radicals attack fatty acid side chains of membrane phospholipids. In this case increase lipid peroxidation resulted in morphological changes in cells of heart, certain chromosomal aberrations and carcinogenesis have also been reported as a result of lipid peroxidation [28]. That the significant increase in MDA resulted in chromosomal aberrations or carcinogenesis could not be ascertain because chromosomal damage was not assessed in tissues like lung and heart that featured histologic changes.

MDA is a lipid peroxidation product formed after free radical attack cell membranes, is a marker of oxidative damage [29]. Determining SOD and Gln-px activities and MDA concentrations reflects the level of oxygen free radical metabolism and the extent of oxidative stress, as SOD and Gln-px are the major antioxidant enzymes that eliminate free radicals and possess antioxidative stress functions. The significant increase in the level of MDA and GSSG in sun exposed rats compared with control suggests increase free radical generation.

The significant increases in serum levels of MDA and GSSG as well as significant decreases for GSH, Gln-px and SOD suggests the involvement of oxidative stress in changes recorded for lung tissues. For example while control rats presented with no histopathological lesions heart and lung presented with mild congestion of the coronary vessel and mild pulmonary congestion respectively. Interestingly, ileum and brain of sun-exposed rats also manifested no visible lesion just like control, an indication of lack of toxic effects of sun rays on these tissues at daily exposure of 4 hours. The lack of morphologic changes in tissues like brain and ileum even when some of the oxidative stress markers were significantly different that is suggestive of free radical generation, may be as a result of the fact that free- radical are highly reactive with very short half-lives, which means they combine almost immediately after their production in situ.

These results are fascinating because another mammalian species- the human are known to be constantly exposed to the sun and it has not be proven that sun exposure causes systemic oxidative stress in human, although that can be theoretically assumed, since UVB is capable of generating free radicals. The results of this study that revealed significant differences in many of the markers of oxidative stress as well as significant damage to tissues may also be associated with lack of significant amount of melanin in Wistar rats. In addition, rats are known for their nocturnal habit and 2012, the year in which the study was conducted has been recorded to be one of the years with the highest level of sun ray/intensity in the past decade.

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