Section A-Research paper
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Effect of U.V. Irradiation on Chemical Profile of mammals Exoskeleton and protective role of Antioxidants

Shweta sharma[1],Anil Kumar Soni[2], Roli Agrawal[3]and Babita Agarwal[1*]

[1]Research Scholar, [1]Department of Chemistry Babu Shivnath Agrawal (P.G) College, Mathura, (Affliated with Dr. Bhimaro Ambedkar University, Paliwal park, Agra, 282004) U.P.

[2]Assistant Professor, [2]Department of Chemistry, Shia Post Graduate College,Sitapur Road Lucknow (Affiliated with University of Lucknow, Lucknow)U.P.

[3]Associate Professor, Department of Chemistry, S,V.College, Aligarh (Affiliated with Dr.Bhimaro Ambedkar University, Paliwal park, Agra, 282004)U.P.

[1]Professor,Department of Chemistry, Babu Shivnath Agrawal (P. G.) College, Mathura, (Affiliated with Dr. Bhimaro Ambedkar University, Paliwal park, Agra, 282004) U.P.

***Author for correspondence: Professor Babita Agrawal**

E mail: babitakanodiya10@gmail.com

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Abstract

The UV component of sunlight is well known to cause serious issues for skin and hair like photoaging, erythema, edema, skin thickening, wrinkling, loss of shine and strength of hair, etc, by triggering the generation of ROS, degradation of proteins, peroxidation of lipids. When the levels of important elements like N, P, Ca, and Mg and crude fat or amino acids were estimated in the hair and nails of pet dogs relying on a normal diet, the concentration of all these elements was considerably lower than the dogs consuming a Vitamin E-rich diet. The data suggest that UV exposure has a negative effect on the concentration of these elements, minerals and amino acids however, the effect can be reversed by the supplementation of an antioxidant-rich diet. The present study help to understand how the aging can reduced by using antioxidant in their regular diet and their positive aspects on exoskeleton of mammals.

Introduction

Skin is the largest organ of the human body and the primary organ of defense [Panich et al., 2016]. It is constantly exposed to the environment and hence significantly influenced by

environmental stresses. Though sunlight is the ultimate source of energy and is necessary for the existence of life on earth,nevertheless, the ultraviolet (UV) fraction of sunlight is deleterious to health.The wavelength of these high-energy electromagnetic waves lies in the range of 380 nm to 10 nm. The ozone layer protects the planet from harmful UV radiation, yet increased UV radiation is one of the environmental challenges that affect people's health globally [Sherry, 2019]. In the presence of atmospheric oxygen, these rays trigger the production of Reactive Oxygen Species (ROS) causing the oxidation of the protein and lipid content of the hair leading to hair damage [Grosvenor et al., 2016; Hoting et a., 1996],premature aging of the skin (photoaging), erythema, skin pigmentation, cancer and several more serious diseases related to the exoskeleton of mammals [Dreher et al., 1998].

The minerals are necessary to build animal tissues, maintain normal physiological processes and also reflect the metabolism of nutrients in the body [Chitturi et al., 2015]. The consistency of the chemical composition of the body is one of the most crucial and obligatory state for proper functioning because variations in the concentrations of chemical elements brought on by environmental, occupational, climatic, or geographical factors are the root cause of various disorders [Avtsyn et al., 1991; Skal'nyi, 1999].Recent research reveals that changes in trace metal levels may affect the incidence and occurrence of chronic diseases like cancer [He, 2011].

The selection of a sample to detect the trace element in the body is an important step and various samples can be used for this purpose [Esteban and Castaño, 2009; Nagornaya and Dubovaya, 2006], however, all the samples are bound with certain advantages and disadvantages. Nails are epidermis-derived body parts mainly composed of keratin-rich protein and accumulate trace metals proportionately to their intake through a variety of methods, including the production of proteins with sulfhydryl groups. Owing to fact nails are considered a useful marker for trace metal analyses and are increasingly being used in clinical trials [Janbabai et al., 2018]. Another skin-derived body part is hairs which are also a metabolic end product. Hairs have a specific potential to demonstrate the body's metal load. The amount of certain trace elements present in hair reveals a balanced mineral content of the body over a long period. Thus, for trace element analysis, both the samples have several advantages*viz.* the sampling is noninvasive, portable, and can be stored for a long time without special conditions; using these samples, both short- and long-term effects can be assessed, and the dynamics of substance accumulation can be determined [Esteban and

Castaño, 2009; Were et al., 2008]. We sought to investigate the effect of the UV radiation present in sunlight on the level of few selected elements in nails and hairs. Hence in the present study dogs (variety: Labrador retriever) were selected to study the effect of UV exposure on the trace elements, crude fat and amino acid profile study in nails and hair samples before and after giving antioxidant rich diet.

Materials and methods

Animals and diet: In the current study pet dogs of the German shepherd variety were used as animal models. All the dogs were healthy ofx-y years age and had a weight range of 10-14 kg. The dogs were randomized and divided into two groups based on diet, one group was getting a normal diet while the other was getting Vitamin E-rich Pedigree. The dogs were given 5% of food according to their body weight.

Sample collection: Nail samples were collected from healthy, disease-free dogs (variety:GermanSephered) which are from a nearby pet seller shop. The nails are cut by a veterinary doctor in a definite proportion by using a sterile volute (veterinary hospital) nail cutter. Samples were collected by cutting the terminal portion of nails and special precaution was taken to avoid damage to the nail plate. Subsequently, the nails were stored in airtight sample bottles (Lapro technologies, Mumbai, India) and used for further analyses.

Determination of the mineral composition of nail samples: The mineral composition in nail samples was determined by using the Atomic absorption spectroscopy technique and U.V. visible spectrophotometer. Firstly, the sample was converted into ash by using a muffle furnace at 550 °C by using ammonium nitrate because it helps to remove other minerals which are not detectable. Then collect ash and treat it with hydrochloric acid then all mineral ion is converted into their chlorides. These chlorides undergo filtration and are analyzed by using PE5000 atomic absorption spectroscopy. Phosphorous is determined by using vando-molybdate reagent calorimetrically at a wavelength of 420 nm. The measurement of other minerals takes place by using U.V. visible spectrophotometer recording their samples at different wavelengths in the range of 200-400 nm. Every ion shows a peak at a certain wavelength in spectra.

Crude fat analysis of hair and nails sample

The crude fat analysis of hair and nail samples from German shepherds was conducted using the Soxhlet extraction method. This method involves extracting lipid material from the

samples using a solvent such as petroleum ether or hexane.To begin, powdered samples weighing 3 grams each were prepared using a muffle furnace. These samples were then placed in separate cellulose thimbles. The thimbles containing the samples were placed in a Soxhlet apparatus, and a solvent was added to the extraction flask. The heating mantle was turned on, causing the solvent in the flask to boil and evaporate. The evaporated solvent vapor condensed in the water-cooled condenser, dripping onto the sample in the thimble. The solvent dissolved the lipid material in the sample and carried it back to the extraction flask. This process continued until the solution in the flask became clear, indicating that the lipid material had been collected.Next, the extraction flask was removed, and the solvent was evaporated using a rotary evaporator to obtain the crude fat. The obtained crude fat was then weighed for further analysis.

Determination protein in hair and nails samples

The collected hair and nail samples are cleansed using a mild detergent to eliminate impurities such as dust and oil. Subsequently, the hair and nails are thoroughly rinsed with distilled water and dried. They are then carefully cut into small pieces and ground into a fine powder using a mortar and pestle.Next, the hair and nail powder are mixed with a buffer solution, such as Tris-Hydrochloric acid, and incubated to facilitate protein extraction. Following extraction, the mixture undergoes centrifugation to separate the supernatant containing the proteins from the hair and nail residue.The protein concentration in the supernatant is determined using a colorimetric assay that relies on the interaction of a dye with the proteins. This assay provides a quantification of the protein content in the supernatant.Subsequently, the protein-containing supernatant undergoes acid hydrolysis, utilizing HCl, to break down the proteins into their constituent amino acids. The resulting amino acids are then analyzed using high-performance liquid chromatography (HPLC). This analytical technique enables the identification and quantification of individual amino acids present in the protein samples.

Results

To conduct this study we selected $N_{2,}$ phosphorous, Mg, and Ca as markers of hair and nail health as these elements have crucial roles in the physiology of the whole body. Nitrogen is an imperative part of proteins of the body, phosphorous promotes the growth and repair of all the cells including skin, hair, and nails. Calcium is important for healthy hair, nails, and the

repair of all damaged tissue, while Mg is directly involved in hair growth and calcium buildup in hairs.

Effect of UV exposure and antioxidant-rich diet on elemental status in hair

Table 1 represents the estimation of elements in hair samples of dogs getting normal and antioxidant-rich diets. In the case of dogs feeding on a normal diet, it was evident from the data that Calcium was the most abundant element, which was quantified as 1204.16 mg/100g hair followed by Magnesium (77.21 mg/100 g hair). Nitrogen (N_2) and Phosphorous (P) were found at the concentrations of 14.17 and 27.68 mg/100g hair, respectively. Interestingly, when these elements were analyzed in hair samples of dogs having an antioxidant-rich diet the quantity of each element was considerably higher (Ca, 1367.18; Mg, 83.55; N_2, 14.36; P, 28.93 mg/100 g hair).

Table 1: Estimation of elements in hair samples

S no	Element	Quantity mg/ 100 g hair	
		Normal diet	Antioxidant-rich diet
1	Calcium	1204.16	1367.18
2	Magnesium	77.21	83.55
3	Nitrogen	14.17	14.36
4	Phosphorus	27.68	28.93

Effect of UV exposure and antioxidant treatment on elemental status in nail

Similar to hairs, the same 4 elements were analyzed in the nails of both groups. In nails, the amount of Ca was highest (469.89 mg/100g) followed by P (78.13 mg/100g). The quantity of N_2 was comparable to hair (14.47 g/100g). The amount of Mg was estimated as 54.63 mg/100g. On the other hand, feeding the dogs an antioxidant-rich diet resulted ina significant increase in the concentration of all the elements as in the case of hair (Table 2).

Table 2: Estimation of elements in nail samples

S no	Element	Quantity mg/ 100 g nail	
		Normal diet	Antioxidant-rich diet
1	Calcium	469.89	473.36

2	Magnesium	54.63	58.96
3	Nitrogen	14.47	15.93
4	Phosphorus	78.13	79.98

Effect of U.V. exposure on crude fat analysis of exoskeleton

The percentage composition of crude fat increases on applying antioxidant rich diet to a research model (German Sephered). The percentage of crude fat in hair and nails before and after giving antioxidant rich diet is as follow (Table 3).

Table 3Variation of crude fat in hair and nails samples respect to antioxidant

S no	**Crude fat**	**Quantity Variation in percentage (%)**	
		Normal diet	**Antioxidant rich diet**
1	Hair	6.2%	7.9%
2	Nails	3.2%	3.7%

In hairs percentage of crude fat raise fastly then nails.

Variationof amino acid in Hair samples with respect to antioxidant

There is a variation take place in concentration of amino acid before and after giving antioxidant rich diet antioxidant effect the concentration of various amino acid like cysteine, methionine, and glutamic acid which decreases with respect to age due to change in metabolic activites.

S.no	**Amino acids**	**Quantity Variation in percentage (%)**	
		Before antioxidant rich diet	**After antioxidant rich diet**
1	Aspartic acid	2.77	3.80
2	Threonine	1 .44	1.58
3	Serine	1.86	1.97
4	Glutamic acid	5.66	7.45
5	Hydroxyproline	0.88	1.89

6	Proline	2.99	4.1
7	Isoleucine	1.36	2.37
8	Leucine	2.95	3.97
9	Tyrosine	3.95	2.84
10	Phenylalanine	1.60	2.61
11	Histidine	1.78	2.99
12	Hydroxylysine	0.10	1.08
13	Lysine	2.64	3.98
14	Arginine	2.22	3.31
15	Glycine	2.99	4.1
16	proline	2.90	3.96
17	Cystine	0.74	2.1
18	Valine	1.78	2.87
19	Methionine	0.83	2.19
20	Alanine	2.52	3.53

The above table represent that the concentration of some amino acid increaseas very significantly after giving antioxidant rich diet such as asparatic, glutamic, histidine arginine, alanine and valine and some amino acids concentration change little bit given as above (Table 4).

Variation of amino acids in nails sample with respect to antioxidants

Table 5: Variation of amino acids in nail sample.

S no	**Amino acids**	**Quantity Variation in percentage (%)**	
		Before antioxidant rich diet	**After antioxidant rich diet**
1	Aspartic acid	2.73	3.76
2	Threonine	1 .24	2.37
3	Serine	1.56	1.77
4	Glutamic acid	4.66	5.26
5	Hydroxyproline	0.89	1.30

6	Proline	2.39	4.4
7	Isoleucine	1.26	1.79
8	Leucine	2.87	3.89
9	Tyrosine	3.85	3.94
10	Phenylalanine	1.72	1.73
11	Histidine	1.68	1.98
12	Hydroxylysine	0.10	1.08
13	Lysine	1.64	1.88
14	Arginine	2.12	2.21
15	Glycine	2.89	3.1 4
16	proline	2.80	2.96
17	Cystine	0.94	1.1
18	Valine	1.68	1.97
19	Methionine	0.73	1.39
20	Alanine	2.12	2.15

The above data represent the variation of amino acids with respect to their antioxidant rich diet. The concentration of asparatic , glutamic , theronine,proline and methionine show large variation in their concemtration then comparision to others.

Discussion

The depletion of the ozone layer is a major environmental problem for the world, due to which there is an enhancement in the UV radiation reaching the earth, which causes several serious problems to humans as well as animals. The hairs and nails are part of the skin which are maximally exposed to UV radiation. Hence hairs and nails face the most detrimental effects of UV radiation along with the skin.The UV fraction of sunlight weakens hair fibers and causes damage. Keratin (the hair protein) and melanin pigments are damaged by UV-B, and the interaction of endogenous photosensitizers with UV-A results in the production of free radicals and reactive oxygen species [Fernandez et al., 2012]. All these reactions ultimately cause serious damage to the skin, nails, and hair.

In recent decades, there has been an increase in awareness of the significance of trace metals in matters relating to human health. By altering enzymatic activities or the permeability of cell membranes, trace metals are advantageous nutritional components that function as

crucial cofactors in physiological processes [He et al., 2011]. Owing to the fact in the current study, we selected pet dogs as study models, and hair and nails as sample tissue to study the effect of UV radiation. In several previous studies, these body parts have been used to determine the trace elements in the biomedical and environmental sciences [He et al., 2011; Sherry, 2019]. The protective role of antioxidants is well documented in the case of hair and is regularly used in cosmetic products due to these properties. Hence, in this study, one group of animals was given a vitamin E-rich diet which is a good source of antioxidants. An improved level of all the tested elements was observed in the animals consuming Vit E rich diet. Previously, a similar study was conducted on human hair by Fernandez et al [2012]. The authors exposed the hair samples to UV light and various parameters like protein and amino acid degradation, lipid peroxidation, color and shine changes, and strength/relaxation properties. All the parameters were worsened after UV treatment, however, when the hairs were treated with antioxidants an improvement in all the tested parameters was observed.The generation of ROS by UV light is supposed to be one of the main reasons for photoaging. The exogenous supply of antioxidants through diet and/or skin pretreatment can inhibit the photoaging process [Petruk et al., 2018].

Conclusion

Like humans, domestic animals are also constantly exposed to UV radiation. The consequences of UV radiation in these pet animals can be correlated to humans also. The data suggested that UV exposure decreased the levels of essential elements and amino acids in hair and nail samples. Interestingly the antioxidant supplement through diet alleviated the negative effect of UV radiation and reduced the chances of premature aging.

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