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Consequences of Hot Air and/or γ - Rays on Hair Hygiene Post the Application of Protein Conditioner in Rats: Urea, Creatinine, Scanning Electron Microscope and Fourier Transform Infrared Analyses

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Abstract:Due to the vast growth in the usage of hair care products, proper evaluation of hair damage is essential for considering the impact of cosmetics or devices used on human health. The objective of the submitted work was to assess the effect of hot air and/ or gamma irradiation following the application of a protein conditioner on the urea, creatinine and hair health. Forty eight albino rats were employed in this work, half of them were females and the other half was males. The animals were divided into eight groups, each of six, three of them were males. Rats in group I were fedfree on normal food and water (control group). Animals in groups II, III &IV were rubbed with protein for once, twice and three times, respectively, every so often was separated by one week duration. Following each treatment, the rubbed area was exposed to hot air from a blow dryer (95°C) for one minute. Rats in groups V, VI &VII were tracked the same previous treatment exactly, and a week later, they exposed to 10Grays (Gy) single dose whole body gamma irradiation. While, for animals in groupVIII, after applying the protein conditioner, they were exposed to10 Gy γ-irradiation without hot air. One week post-irradiation, rats in all groups were anesthetized and blood samples were collected and serum was separated for urea and creatinine analyses. Then, the hairs cut from the protein applying area and subjected to Scanning Electron Microscope examination (SEM) in addition to Fourier Transform Infrared spectrometry (FT-IR).The obtained data illustrated that urea and creatinine concentrations were increased significantly in both female and male treated groups as compared to the controls .On the other hand, hair density decreased, and alopecia was induced specially, the animals received protein conditioner plus heat and irradiation three times. As indicated, for hair damaged by hot air, in most cases, the amino acids of the cuticle are altered. The hair was very susceptible to chemical changes as a result of exposure to hot air and /or γ-radiation.In conclusion, there is no protein -based conditionerhair smoothing product that can be considered completely safe. It is worth to state that, it's time to give serious considerations for stopping these treatments using the available protein products. Even if there is not noticed any negative effects as yet, it doesn't mean that human is immune but cumulative exposure can increases the risk.

Keywords:- *Hair conditioner, hot air, γ- rays, urea, creatinine, FT-IR, SEM.*

I. INTRODUCTION

Chemical and physical properties of human hair are the subject of a remarkably wide range of scientific investigations due to their importance to the biomedical, cosmetics industry and forensic sciences. [1]. During the course of criminal investigations, the identification and comparison of human and animal hairs can be helpful in demonstrating physical contact with a suspect, victim, and crime scene. [2].

It is well known that human hair and all other mammalian hair fibers belong to a group of fibrous protein, known as α- keratin (90%), and a small amount of lipid (1–9%). [3] The principle protein component of hair is the cysteine rich keratin, which is composed of 18 amino acids and assembled into heavily melanized fibers that form up to 95% of hair fiber volume. These protein components and structural organization of keratin contribute to most of the characteristic properties of hair.[4].The hair shaft of mammals is divided into three main regions: cuticle, cortex and medulla. [5]. Cosmetic chemical treatment processes affect the chemical structure of α- keratin fibres via targeted the bonds that provide stability to the fiber. However, there are two main ways for a substance to penetrate the hair fiber depending on the size of the molecule, trans cellular and intercellular diffusion. Trans cellular diffusion involves epicuticle, A-layer, exocuticle, endocuticle and is much harder path way because of the high cross-linked regions. Intercellular diffusion involves the intercellular cement, and it is the preferred route for large molecules because the low-sulphur and non-keratin proteins are more easily swollen. [6].The important linkages between adjacent keratin chains is the disulfide bond (-S-S-) that through breaking and reforming of these bonds hair can be reshaped. [7]. In contrast to skin or other cells in the body, hair fiber does not possess its own biological protective and repairing mechanisms against the impact of environmental effects. [8].

Cosmetic hair conditioner consists, mainly, of cationic surfactants, fatty alcohols, silicones and water in addition to set of amino acids. The chemical effect of conditioner is based mainly on cationic surfactants. At one end of every cationic surfactant molecule is a positive charge that binds

to the negative charge of a hair strand, primarily through Van der Waals attractions. [9]. The attraction is so strong that the surfactants completely surround the strand and cover the cuticle flakes, like a customized hair envelope. [10].

All protein conditioners contain formaldehyde and/or formaldehyde-producing ingredients including formalin, methilene glycol, glyoxylic acid and others. These ingredients turn into formaldehyde when they break down during the heat application. Exposure to formaldehyde can cause irritation of the skin but, there is valuable data support a link between long- term of formaldehyde exposure and the development of leukemia.[11].

The present work is a trail to manifest the hazards effect of using protein conditioners on hair hygiene, urea and creatinine under exposure to hot air and/or γ- irradiation. SEM and FT-IR tools were performed to follow the impacted damages due to one of the mostly used protein conditioner in Egypt.

II. METHODOLOGY

A. Animals groups

The study was conducted in accordance with the guide lines set by the CIOHS & ICLAS International Guiding Principles for Biomedical Research involving animals,

Group	Description
Group I	Negative control .Rats were fed on normal food and water.
Group II	Animals were subjected to single treatment with protein conditioner followed by hot air.
Group III	Rats in this group were treated with the hair conditioner and hot air twice separated by a week duration.
Group IV	Rats in this group were treated with the hair conditioner and hot air three times separated by a week duration.
Group V	Animalswere subjected to single treatment with protein conditioner followed by hot air then, after one week, to10 Gy single dose γ-irradiation
Group VI	Rats in this groupwere subjected to twice treatments with protein conditioner followed by hot air separated by week duration. After an additional week, rats were received 10 Gy single dose γ-irradiation.
Group VII	Animals were subjected to three treatments with protein conditioner followed by hot air separated by week duration. After an additional week, received 10 Gy single dose γ-irradiation.
Group VIII	Animals included in the group were only exposed to the 10 Gy single dose directly post conditioner application for one time.

Table 1. Design of the animal groups

2012, which are in accordance with the Guide for the Care and Use of Laboratory Animals. (Eighth Edition, 2011, published by The National Academies Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) . This guide was approved by the Ethical Committee at National Center for Radiation Research, Egyptian Atomic Energy Authority, Cairo, Egypt (NCRR- EAEA).The study was carried out on forty eight adult albino rats (weighting 200±10 gm),one half was females and the other was males. The animals were supplied by the Laboratory of Animal House, Nuclear Research Centre (NRC), Egyptian Atomic Energy Authority. They were housed under conventional conditions on sawdust (free of toxic compounds). The mean ambienttemperature in the housing facility was 28ºC (range 26 – 32ºC), and the mean relative humidity was 60% (range from 50 – 70 %)with 12 hours light: 12 hours dark cycle. The animals were freely fed on a normal rodent pallets diet and clean water offered by ad-libitum throughout the whole experimental period. They were divided into eight groups, each of three males besides equal number of females. The animals groups were subjected to the various treatments according to the table 1.

B. Treatment

About half gram of a first grade imported protein conditioner, supplied by local market, was applied on ≈ 3x3cm rat's dorsal area and rubbed for nearly 3 min. At end of the application time, the massaged place was exposed to hot dry air, from a blow dryer, for an additional one minute. Measured temperature at the rubbed spot was about 95 ºC. Animals were placed in ventilated metal containers before subjecting to whole-body γ- radiation.

C. Hair and Blood samples collection

One week later, posts to the all planed treatments were performed, rats were anesthetized by diethyl ether and blood samples were drawn from the retro-orbital venous plexus in glass tubes. Hair samples were cut from the treated spot and sent to spectroscopic examinations. The blood samples were collected and transferred into cold tubes and centrifuged for 10 minutes at 3000 rpm.The sera were separated and frosted in deep freezer at -20 ºC ready to be subjected for urea and creatinine analysis.

D. Scanning Electron Microscope Examinations (SEM)

The hair fibers before and after the various treatments were sputtered with gold and the fine structures of the films was observed using High Resolution Transmission Electron Microscope (HRTEM) SEM-Quanta FEG-250, Holland and imaged at an accelerating voltage of 20 kV.

E. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra were obtained for hair by Nicolet™ iS™ 10 FT-IR Spectrometer, USA. The pressed pellets were made by mixing hair and Potassium bromide (KBr) at an approximate ratio of 1:100 hairs to KBr. The mixture was ground vigorously in order to make the hair pieces smaller and ensure uniform distribution. Exposure to the atmosphere was avoided as much as possible to prevent water absorbing to the pellet.Spectra of the prepared plate were obtained in the transmission mode. They were obtained in mid IR region range (400–4000 cm^{-1}).

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F. Biochemical Analyses

Urea and creatinine were measured in serum by kinetic and fixed endpoint method using commercially available kits according to the techniques of Orsonneau et.al,[12] and Fossati, et.al, [13], respectively

III. RESULTS

A. Morphological features hair post various treatments

Figure (1) illustrated pair of photographs for the treated dorsal spots in rats' dedicated well defined alterations in hair hygiene after the various treatments post application of protein conditioner. Such changes in hair features increased with the repetition of the treatments and applications, where the hair was ungroom, fig.1 (a) and its density had decreased, fig.1 (b), accompanied with alopecia. Fig.1 (c &d).

B. Concentrations of serum urea and creatinine under various treatments

It is clear from Table (2) that, there are significant increases in male rats' serum urea contents by repeated applications of conditioner to record 56 ± 0.58 mg/dl after three times of conditioner application followed by hot air each time and 10 Gy single dose gamma irradiation post the last treatment (Group VII). This elevation was computed by more than 37 % compared to control group. Also, raises in creatinine serum contents were manifested to account 0.59 ± 0.006 mg /dl for the same group, i.e. Group VII, with nearly 60% increase relative to the control one.

For females, the same trends were disclosed, where urea and creatinine contents in serum increased with time after time treatments and the Group VII showed the highest concentration compared to the other groups. Table (2). Therefore, post the three times application of the protein conditioner and hot air followed by single dose of γ-rays, urea and creatinine contents recorded 34.6 mg/dl and 0.566 gm/dl, respectively.

However, it is worth to mention that , for male and female rats , the animals exposed only to 10 Gy single dose gamma irradiation post the one time conditioner application ,namely, Group VIII, showed urea and creatinine contents greater than the groups that not irradiated (Groups II,II&IV) but less than the groups that irradiated after hot air application (Groups V,VI & VII). .Table (2).

It is obvious from Fig (2) that, there were no significant differences in urea contents [A] or creatinine concentrations [B] for males or females animals under various treatment conditions.

C. SEM Examinations

Rats' hair in control group showed undamaged fibers. The surface of the hair is smooth uniquely shaped with discriminated scales and nearly spaced apart.Spinous or Petal-like scales are triangular in shape and projecting from the hair shaft. (Fig 3).

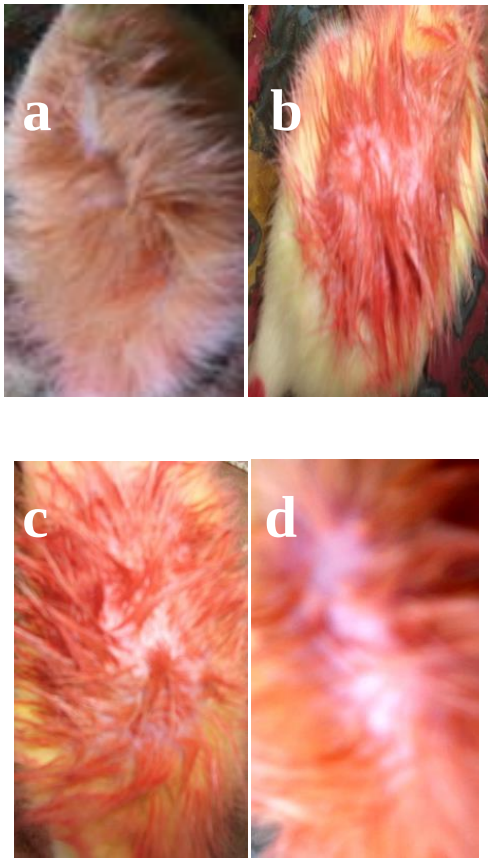

Fig 1:- Photographs for the treated dorsal spots in rats post various treatments

Figure (4) illustrated the impact of hot air and /or gamma irradiation post protein conditioner appliaction on hairs of rats in various groups. Reapeted treatments caused severe cuticle damage. The hair fiber surface showed cuticle disintegration with missing cuticle pieces and jagged cuticle layers, fig.4 (a-d), associated shafts frizzing, fig.4 (e &f), with fusions of some cuticle cells, fig. 4(g), and hair dryness.Fig.4 (h).

D. FT-IR Spectroscopy

FT-IF spectroscopy had been used to follow the chemical damage of individual hair fibers by comparing the spectra obtained for a normal untreated hair and hairs that had been subjected to the different treatments.

The FT-IR spectrogram for hair from untreated animals, Fig (5), showed peaks at the vicinity 3277 cm^{-1}-3050 cm-1 (multiple broad band's), 2917 cm-1, 2825cm-1. These bands can be attributed to stretching vibration of N-H in amide A; asymmetric and symmetric vibrations of CH_2, respectively

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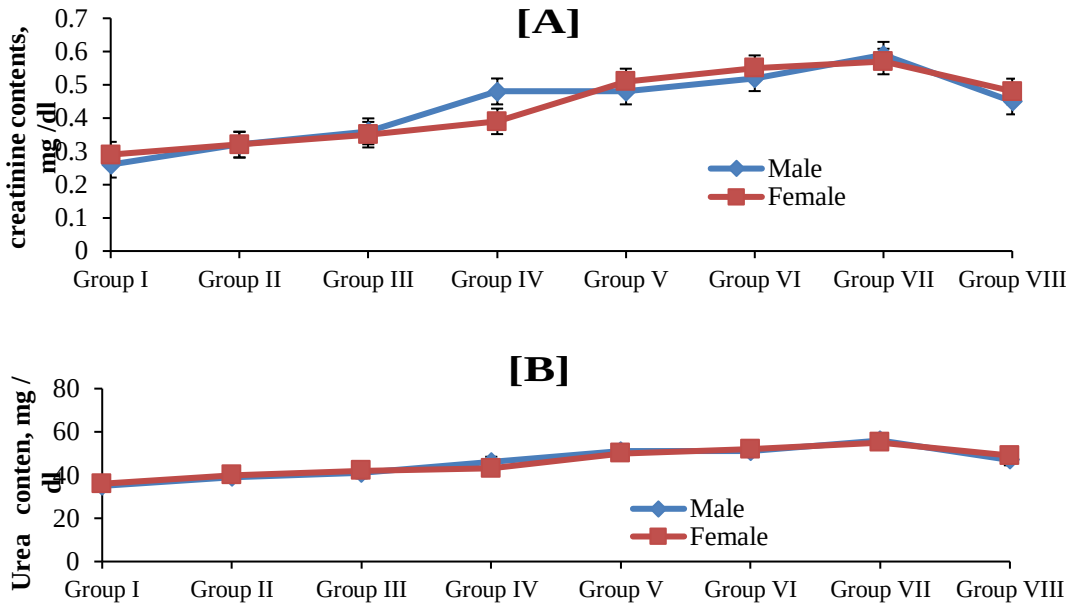

Fig 2:- Serum contents of urea [A] and creatinine [B] in male and female rats in different groups.

Groups	Male		Female	
	Urea (mg/ dL)	Creatinine (mg/ dL)	Urea (mg/ dL)	Creatinine (mg/ dL)
Group I	35.00[a] ± 0.58	0.26[a]± 0.01	35.67[a]± 0.33	0.29[a]± 0.01
Group II	38.67[a] ± 0.33	0.32[a]± 0.01	39.67[a] ± 0.88	0.32[a]± 0.01
	10.3 %	18.8%	10.0%	9.4%
Group III	40.67± 0.67	0.36± 0.01	41.67± 0.88	0.35± 0.01
	14.6%	27.8%	14.3%	17.1%
Group IV	46.00± 0.58	0.48± 0.01	43.33± 0.33	0.39± 0.02
	23.9%	45.8%	16.3%	25.6%
Group V	50.67± 0.67	0.48± 0.011	50.33± 0.88	0.51± 0.01
	31.4%	45.8%	28.0%	43.1%
Group VI	51.00± 0.58	0.52± 0.01	52.00± 0.01	0.55± 0.01
	31.4%	50.0%	30.8%	47.3%
Group VII	56.00± 0.58	0.59± 0.01	54.67± 0. 33	0.57± 0.01
	37.5%	55.9%	34.6%	49.1%
Group VIII	47.00± 0.58	0.45± 0.003	48.67± 0.67	0.48± 0.006
	25.5%	42.2%	26.5%	39.6%

Table 2. The Variations in urea and creatinine concentrations of rats' seraunder various treatment conditions
Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically significant.
The percentage values calculated relative to the control ones in each group .

- Group I: control group.
- Group II: rats exposed to one time protein conditioner application followed by hot air.
- Group III: rats exposed to two times protein conditioner application followed by hot air.
- Group IV: rats exposed to three times protein conditioner application followed by hot air.
- Group V: rats exposed to one time protein conditioner application followed by hot air then single dose γ-rays.
- Group VI: rats exposed to two times protein conditioner application followed by hot air then single dose γ-rays.
- Group VII: rats exposed to three times protein conditioner application followed by hot air then single dose γ-rays.
- Group VIII: rats exposed to one time protein conditioner application followed by single dose γ-rays.

. The band near 1637 cm^{-1} can be due to stretching vibration of C= O in Amide I, while the broad band assigned at 1550-1500 cm^{-1} can be refer to stretching vibration of CH and bending of NH in amide II. Peaks near 532 , 1080, 1229, 1397, and 1449 cm^{-1}, can be consign to : out-of-plane CO bending ; S-S bonding, CN stretching and NH bending in amide III ; CH_3 bending and CH_2 vibrations , respectively.IR bands arising from the S–H stretching mode of cysteineresidues usually occurs between 2520 and 2600 cm^{-1}, but the strong hydrogen bonding between an S–H group and apeptide carbonyl leads to a shift of thiscorresponding IRband to lower frequencies ,namely, near 2350 cm^{-1} .Fig (5) .[14].The differences in the FT- IR spectra for the rats' hair post the various treatments showed an appearance of new peaks at vicinity 3850-3300, 2360-2340 and near 1175 cm^{-1}. These peaks can refer to the O-H bonded to water, O-H and NH_3^+ stretching, in addition to cysteic acid formed by oxidation of cystine, respectively. On the other hand, the peak at 1080 cm^{-1} that attributed to sulphur - oxygen bonding was disappeared. Table (3).

IV. DISCUSSION

In contrast to skin or other cells in the body, hair fiber does not possess its own biological protective and repairing mechanisms against the impact of environmental effects. Hair is very susceptible to chemical changes that occur under the stress of heat and irradiation exposure,[15], the hair – was once shiny and smooth – became frizzy, dry, clumped and yearning for repair.

Most of hair cosmetics contain significant huge amounts of chemicals mainly, formaldehyde. In the present study, urea and creatinine levels, which are significant indicators for renal function, were increased among the all treated groups. Such results were suggested that the formaldehyde contained in protein conditioner was penetrating the affected skin by the hot air facility and infiltrated into blood stream promoted kidney malfunction in addition to elevated contents in urea and creatinine that generated from the skin under the stress of hot air and/or high dose of irradiation. It should be stated that, the serum contents of urea and creatinine can be used as relatively simple method to estimate chemical induced blood flow changes in human skin.[16]. This is in agreement with the data of Zararsiz et al., [17] and Kunak et al. [18], who reported that strongly, suggested impaired in kidney function due to the exposed to the formaldehyde of the protein conditioner which penetrate the skin and affect more than one organ in the body. Zararsiz et al., [17, 19], Zararsiz et al., and Dorairajan [20] were confirmed this fact as they reported that formaldehyde caused oxidative injury by impairing anti-oxidant defiance mechanisms in the kidneys which indicated by decrease in glutathione peroxidase and superoxide dismutase activities and increase in malonaldialdehyde (MDA) levels. According to the results obtained, there was an increase in serum urea in animals exposed to the one of the ingredients of protein conditioner, namely, formaldehyde. This is in agreement with the data of Ramos et. al., [21], who reported that, oxidation of formaldehyde to formic acid, is catalyzed by various enzymes such as NAD-dependent dehydrogenase formaldehyde, xanthine oxidase catalase and peroxidase. Increased in serum levels of creatinine, also, strongly suggested impaired in kidney function due to the exposed to the formaldehyde of the protein conditioner, [18], which penetrate the skin and affect more than one organ in the body, as previously stated. [22]. On the other hand, İnci et al., [23], attempted to elucidate the severe nephrotoxic effects of formaldehyde on the histopathological renal tissues. He reported that application of formaldehyde impaired

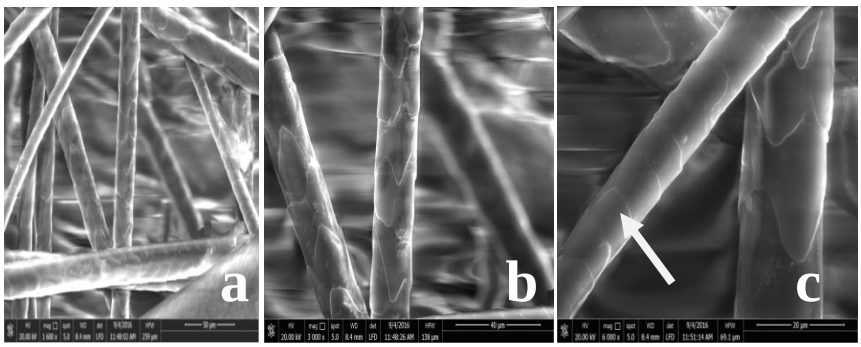

Fig 3:- SEM micrograph for hair for rats in control group showed the normal coronalscales of rats' hair (a-c)

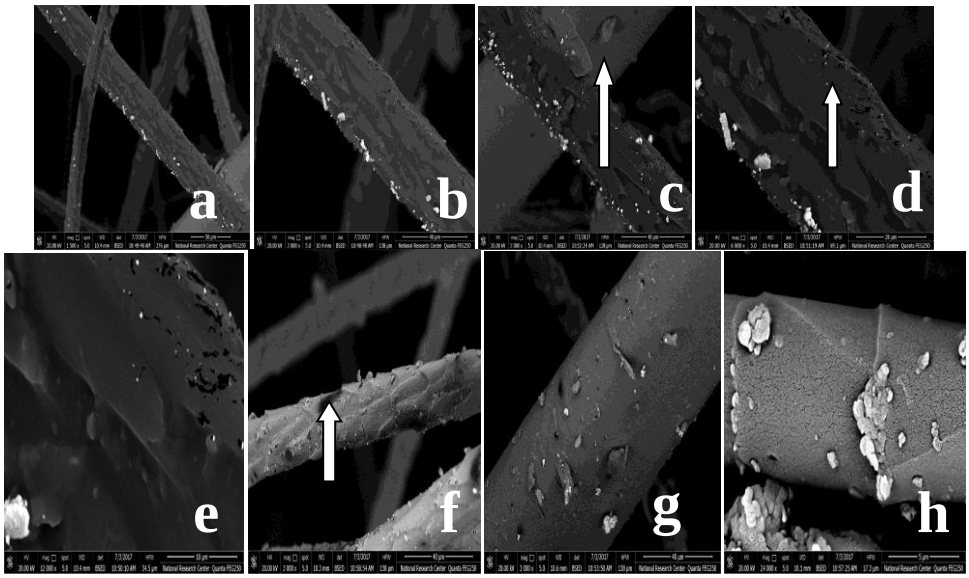

Fig 4:- SEM micrograph for hair from rats subjected to different treatments

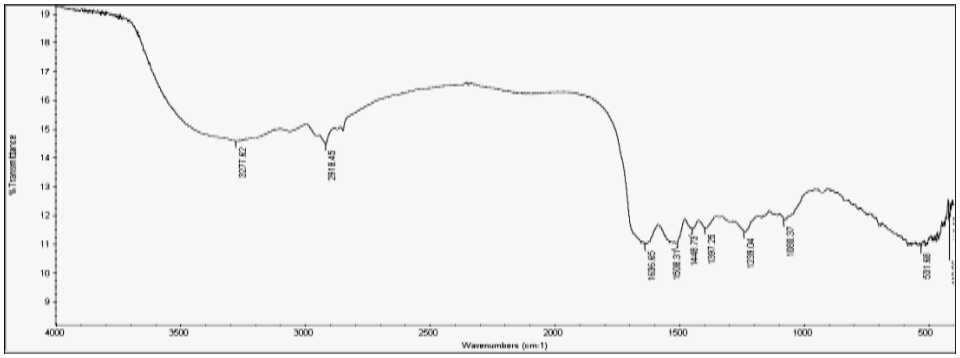

Fig 5: FT-IR of the hair from control group (untreated rats)

Group / Bands ,cm^{-1}	C	Male							Female							Assignment
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	
3850-3300	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	OH, bonded H_2O
3285 -3050	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	γ NH, amide I&II
2919	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	γ_{as} CH_2 asym. Stretching
2850	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	$\gamma_s CH_2$, sym. Stretching
2360-2340	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	OH& NH_3^+ , Stretching
1655	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	γ C=O, amide I
1540	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	γCH_2& δ NH
1450	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	δasCH_3 & γCH_2,
1400- 1390	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	δs CH_3
1240-1230	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	N-H & C-N interaction , Amide III
1174	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	Cysteic acid, cystine oxidation
1080	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S-S bonding
670 - 530	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	Out-of-plane CO&NH bending

Table 3. Characteristic FT-IR bands for the rats' hair subjected to various treatments.

C: Group of control rats.
1: Group of rats exposed to one time protein conditioner followed by hot air.
2: Group of rats exposed to two times protein conditioner followed by hot air.
3: Group of rats exposed to three times protein conditioner followed by hot air.
4: Group of rats exposed to one time protein conditioner followed by hot air then single dose γ-rays.
5: Group of rats exposed to two times protein conditioner followed by hot air then single dose γ-rays.
6: Group of rats exposed to three times protein conditioner followed by hot air then single dose γ-rays.
7: Group of rats exposed to one time protein conditioner followed by single dose γ-rays.

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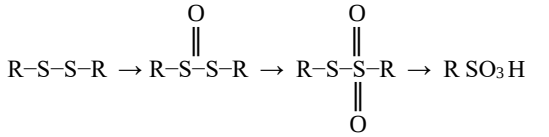
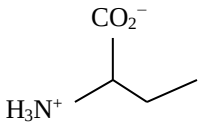
Glomerular patterns and thickened tubular and glomerular basal membranes. In addition to congestion of the intratubular vessels, vacuolization and dilatation in distal tubules.In another study b\]]]Zararsiz et al. al., [19] whofound that formaldehyde application led to glomerular and tubular degeneration, tubular dilatation and congestion. Mammalian hair fibers belong to a group of fibrous protein known as α- keratin. The identifying trait of keratin is the presence of large amounts of cysteine, a sulfur-containing amino acid, which is occupying about 14-24 percent cysteine [4, 10]. Keratin protein chains are very complex due to the multiplicity of the cross-linked protein molecules. One of the most important linkages between adjacent keratin chains is the disulfide bond (-S-S-) that makes the keratin very stable and extremely resistant to biological and chemical degradation.[7].On the other hand, keratin is very reactive, as cystine can easily be reduced, oxidized, and hydrolyzed. [4]. In the present study, SEM topography examination demonstrated that, thermal treatment causes severe cuticle damage. The hair fiber surface showed cuticle disintegration with missing cuticle pieces and jagged cuticle layers. Also, there was a fusion of some cuticle cells, hair shafts frizzing and dry. Cosmetic chemical treatment processes affect the structural chemistry of α- keratin fibres by targeting the bonds that provide stability to the fibre.Also,various abnormalities in the hair and hair follicles caused by heat and irradiation have been reported to be associated with structural re-arrangement and chemical modification in hair keratin. The hair appearance depends on the health of cuticle, when it is strong and healthy; the hair appears to be strong and healthy.These results are in agreement with the study conducted by [1, 9] who suggested that hair has been damaged and the cuticle cells distorted due to the frequency or repeated long term of thermal treatments as hair drier and hair iron. Also, dry hair fibres were damaged with often use of the heat iron for short period (10 seconds) that lead to disintegration, cracking and scale edge fusion of the surface cuticle cell . Miranda -Vilale et.al., [24], illustrated this criteria in his report that closed cuticle acts as protective shield against harmful environmental elements, but when cuticle scale are raised, substances can be deposited in their structure causing harms to the hair.Once the cuticle is damaged, hair breaks easily since there is no protection for the cortex. It should be notified that, degradation of amino acids such as tryptophan, are known to occur from sun exposure also. [25].Hair damage due to thermal treatments has been researched, since hair dryers and hair irons are frequently used to dry hair or to set up hair styles. [1]. Palma et. al., [4], reported that,hair damage was dose-dependent for exposures between 0 and 10.0 Gy. Hair is very susceptible to chemical changes that occur with exposure to gamma radiation. γ -rays can cleave the disulfide bonds and decomposes tryptophan in hair. The numerous disulfide bonds formed by cystine are responsible for the great stability of keratin. On the other hand, keratin is very reactive, as cystine can easily be reduced, oxidized, and hydrolyzed. [4]. Therefore, it is assumed that, the application of protein conditioner followed by hot air and/or gamma irradiation treatments have potential impact on the rats' hair.

The transmission of the bands at 1175 cm^{-1} is indicative of disulphide oxidation of cystine in keratin and corresponding to cysteic acid and sulfonates. This band can be referee to the asymmetric S=O stretch. [26].According to Cardamoneet al.,[27], the presence of the oxide forms of sulphur is significant for determining the extent of oxidation, assuming that oxidation of the disulfide bonds occur by way of monoxide-to-dioxide, to full oxidation with the formation of cysteic acid or sulphonic \\\acid. A broad hydroxyl and NH multiple peaks (3850-3050 cm^{-1}; and near) due to water in addition to primary amine is clearly observed for all the treated samples. New additional sharp peaks appear at vicinity of 2360-2340 $cm^{-1.}$ Both alterations may be due to the breaking of amino acid chains caused by various treatments. The excitation energy of tryptophan aromatic residue disrupts a neighboring disulfide bridge, which in turn leads to altered structural integrity and stability. The disruption of S-S crosslinks in hair induced by radiation may occur not only with SH groups as an end product, but rather through the oxidation leading to the formation of cysteic acid, $CySO_3H$. The oxidation of cystine can be proposed as follows:



Cysteine Cystine monoxide Cystine dioxide Cystic acid

Where R is



For important, several publications demonstrating that biological effect of UV-radiation in dose range 10 - 20 J/m^2 can be significantly more damaging than of 7 or 10 Gy gamma irradiation. [8,28].

V. CONCLUSION AND RECOMMENDATION

The overall conclusions were that permanent application of protein conditioner is highly destructive. The oxidative damage of hair is mainly due to successive treatment and environmental exposure. The prolong exposure to the chemical ingredients in conditioners present a risk of developing cancer including blood cancer.

Without others worthy to be counted, the most favourable rout for improving the hair health and keeping it naturally shining, is through the blood stream. Incorporating the man's diet with high level of natural amino acids and supportive nutrients, every day for adequate periods, hair, will generally recover its shiner, brightness and healthy without application of any artificial protein conditioner. Authors hope to consider going the natural route, which may require a little more effort, but it well knows that hair will look better minus the serious short- and long-term damage to hair and health.

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