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Activities of Glutathione peroxidase , GR, SOD, GSH, LDH and ALP with UV Nano ZnO Sunscreen

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Absract: The UV absorption by thin film made of Sunscreen (Nano ZnO), confirmed the absorption with wide range epidermis and dermis were significantly observed. The change in the activity of GR,glutathione peroxidase ,ALP, total Protein ,LDH, AP, were observed in the serum which shows the effect of UV and Nano sunscreen protective action.

Key Words: NanoZnO,Sunscreen,Glutathione,Glutathione reductase

Zinc oxide have been used by cosmetic industry for many years because they are extensively used as agents(absorbs and /or scatter) the ultraviolet radiation .In the most UV-attenuating agent is formulated in which the metal oxide nano-particles are incorporated into liquid media or polymer media are manufactured, such as sunscreens and skin care cosmetics.(*Al-Hilliand* et al., 2006).Scientists have proven that ultra violet A (UVA) radiation is a major culprit in photo-aging and skin cancers (*Fairhurst* et al., 1997). Inorganic UV absorbers (ZnO) have many desirable characteristics such as along history of topical use, low irritancy, broad-spectrum absorption and high photo-stability. Because of these characteristics Zinc oxide has long recognized for its medicinal properties as an anti-irritant and astringent as well as its UV blocking properties, in sunscreens (*Shore* et al., 1990).Bulk materials may show novel properties when they become nanosized and there is increasing exploitation of these novel properties. Two principal factors cause the properties of nanomaterials to differ significantly from bulk materials: increased relative surface area, and quantum effects.

With constant mass a decreased particle size results in increased total surface area. (*Nel* et al., 2006) ;(*Oberdörster* et al., 2005); (*Hoet* et al., 2004). (*Kreyling* et al**.,** 2006). The resultant larger surface area

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causes surface chemistry to become increasingly important; hence smaller particles may exhibit greater biological activity per given mass compared with larger particles. In other words, the vast amount of a reactive molecule species located only on the surface of insoluble particles and of particle cores (remaining after dissolution of the soluble components) may be the ultimate metric determining adverse outcomes, although this molecule may only add a small fraction to particle mass.Till 1750-1850, zinc oxide and zinc sulfide were formally used in medical treatment in may developed countries in Europe, which leads to increasing study and research on zinc oxide. ZnO may well be used as foodstuff. ZnO is not poisonous at all.

To understand the synthesis-structure-performance relationships of the supported catalyst, it needs to fully characterize their structure and more importantly their structural evolution during the catalytic reaction. Analysis of individual nanoparticles by various techniques such as XPS alternatively known as XRD, TEM, AFM, UV_VIS Spectroscopy and IR spectroscopy has been used.

Some cosmetic products use mineral-based materials and their performance depends on particle size. In sunscreen products, mineral nanoparticles (e.g. titanium dioxide and zinc oxide with particle size in the order of 20nm) are efficient UV-filters. They transmit, reflect and scatter the visible part of the solar radiation while they strongly absorb in the UV region. These mineral UV-filters consist of micron-sized aggregates, which are composed of nanosized primary particles. The surface of these nanoparticles may be treated with an inert coating to improve their dispersion in sunscreen formulations and to prevent photocatalytic activity (*SCCNFP*, 2000).

MATERIAL & METHODOLOGY

Nanoparticles of ZnO were prepared by Sol Gel Method. Biochemical estimation(LDH.,AP.,ALP.,GSH,GR.SOD) were taken before and after application of Sunscreen(nano ZnO particles)application and observed irritability, sensitivity and elasticity of skin. Male/Female Albino rats 1-6 week (150-200mg body weight) old pathogen free were procured from D.A.V.V. animal house. They were supplied with a standard mouse diet. Experimental Albino rats were housed 2-5 animals/cage with a 12 hour light/dark cycles, isolated from other Albino rats, week prior to each experimental protocol.

First group of animal is divided into five groups: control, Group a -UV treated for 45sec.-1 min.,Group b-UV treated for 15 sec.,Group a'-Sunscreen applied and UV treated for 45 sec.

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-1 min.,Group b'-Sunscreen applied and UV treated for 15 sec.For each group 5-6 male Albino rats maintain.

Synthesis of Sunscreen:

Nano ZnO was prepared by the process of Sol Gel method. The following is the process for the preparation of the nano ZnO *(Itoh* et al., 1993).

- 21.9 gm $ZnCl_2$ dehydrate were dispersed in 155 ml 1-Propanol and stirred on magnetic stirrer & clear solution was observed.
- Ammonia gas was passed and ppt was observed.
- The ppt was filtered .and further proceed the clear solution.
- Distilled water was added to drop wise in clear solution was formed from above process & ppt was observed. A white gel like precipitate which disintegrates within in a few minutes and yielding an optically clear sol.
- The volume of the sol was reduced to 50 mL by evaporation and autoclave resulting Nano ZnO which finally purified by calcinations.
- Purification was carried by the Calcinations up to 500 ^{0} C.for 5-6 Hr.
- Clear white Nanoparticles of ZnO were formed.

(21.9 gm)$Zncl_2$ +(23.12ml) CH_3CH_3=CHOH □ $(CH_2=CH_2)Zn$ ……..(1)

$$(CH_2= CHO)Zn \xrightarrow{+NH_3,+H_2O,-NH_4CL} Zn(OH)_2 \quad \text{……..(2)}$$

$$Zn(OH)_2 \xrightarrow{\text{Heating 500°c}} ZnO \quad \text{……..(3)}$$

Sunscreen –Preparation Method:

Sunscreens Preparation is based on *David A .Kartz* 2005.

A process for incorporating ZnO particles in a cosmetic composition comprised of solid asymmetric particles, comprising: Melting solid asymmetric particles to form melted fatty acid; Adding un-coated nano ZnO particles to melted fatty acid to form a mixture of ZnO and fatty acid; Heating mixture to a temperature of less than about 80°C for about 5 to about 10

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minutes; Cooling said to a temperature of about 50°C thereby quenching any reaction between ZnO and fatty acid.

Irradiation:

Prior to irradiation, dorsal area of 4cm^2 was shaved. Albino rats were then irradiated 24 hours later for 15 to 45sec.-1min range (*Taylor* et al., 2002 &*O'Riordan* 2004) .Control group was maintained in separate cages. Experimental Albino rats were kept in wire cages designed to hold individuals Albino rats expose to radiation and ears were protected with white labeling tape during exposure for 0, 15 and 45 sec.-1 min range of UV-B. Ultraviolet irradiation was accomplished using one CH lightning U.V. Tube F 15T18/GL and that emit wavelength between 270 & 390nm with a peak emission at 313nm. Light intensity was determined before and after every exposure using UV-B detector. Intensity remained consistent during exposure ranging from 0.28 to 3& averaging 1.6 joule/cm^2 for all experiment readings and exposure occurred 20cm from the source (*Akram* et.al.2005).

Treatment: Acute Dermal Toxicity Test:

Test substance: Nano ZnO/sunscreen

Concentration:

(a) The test substance was prepared as a 50% w/v paste in distilled water.
(b) Sunscreen prepared.

Dose:

(a) Single dose of 200mg/kg applied uniformly of approximately 10% of the total surface of the animals 10% of the total surface area.

Sunscreen applied before 30min. exposure (approx. 2mg). from the source (*Akram* et.al.2005).

Biochemical Estimation:

1 Colorimetric Method of *King* (1959, 1965) for LDH:

Procedure:

Pipette 1ml of buffered substrate and 20 □l serum (or 0.1ml of serum diluted 1 to 5 with water) into each of two tubes. Added 0.2ml of distilled water to one (the blank) and place both tubes in a water bath at 37°C. Allow to reach the temperature of the bath. Then the other

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tube (the test) adds 0.2ml NAD solution and shakes to mix. Exactly 15mins. After adding the NAD add 1ml of the dinitrophenylhydrazine reagent to each, shake to mix and left in the water bath for a further 15 min. Then remove from the bath and to each add 10ml 0.4 N sodium hydroxide and read at 440 mill microns within 1 to 5min. of adding the hydroxide.It is necessary to add $NADH_2$ in preparing the standard curve since this substance gives a color with dinitrophenylhydrazine at 440m□ (*Kin*, 1960b). NAD also does, but in lactate substrate the absorption of NAD dinitrophenylhydrazone is small (0.015).

2 Estimation of Serum Alkaline Phosphatase: Method of Kind and Armstrong (*King and Armstrong* 1984; *King* et al., 1987, 1942):

Procedure:

Pipette 6ml of the buffer substrate into a test tube and place in the water bath at 37°C for a few minutes. Added 0.3ml of serum, preferably without removing from the bath. Mixed and cork and allowed to remain in the bath exactly 15min. Then remove and immediately add 2.7ml of the diluted phenol reagent. At the same time set up a tube for the control containing 6ml of substrate and 0.3ml of serum to which is added immediately 2.7ml of diluted phenol reagent. Mixed well in both cases and centrifuge. The result is expressed in mg. of phenol liberated by 100ml of serum in 15min. at 37°C each unit corresponding to the liberation of 1mg. Of phenol per 100ml of serum is calculated.

3 Acid Phosphatase activity also observed by King's method. Observation were Compared the reddish brown color immediately at 510 nm.

4 Biuret Protein Assay: Protein estimation is done by this method.

5 Determination of Tissue Glutathione:

The glutathione was estimated by the method of *Beuter* et al., (1963) This method is based on the development of a relatively stable yellow color with 5,5'-dithio bis-2, nitro benzoic acid (DTNB) (Ellman's reagent). Ellman's reagent reacts with GSH to produce a colored ion.

6 Assay for superoxide dismutase:

The enzyme superoxide dismutase[SOD] is specific to dismutate $0_2^{\cdot-}$ to H_20_2 and is therefore described as a superoxide quencher. The ability of the enzyme to inhibit the autooxidation of pyrogallol in presence of EDTA was used as a measure of SOD activity (*Marklund* et al.,

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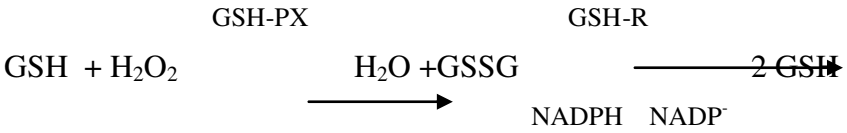
1974).SOD activity was measured in the erythrocyte lysate prepared according to the method of *Mccord* in the supernatant of tissue homogenates prepared in 0.1 m phosphate buffer ph 7.4. The reaction mixture [3 ml] contained 50mm Tris-HCl buffer ph 8.2, 2 mm EDTA, 0.2 MM pyrogalloland 5 – 20 µl of homogenate or erythrocyte lysate. The reactions was started by the addition of pyrogallol and an increase in absorbance was recorded a 420 nm for 10min in a spectrophotomer [Shimadzu UV– 160 A]. A blank was performed before each sample without the additional of the enzyme preparation.

One unit of the enzyme activity is the 50% inhabitation of the rate of the autoxidation of pyrogallol as determined by the change in absorbance at 420 nm/min. The activity of SOD is expressed as units/mg protein and units/Mg of skin. The assay was performed in duplicate in a two fold concentration range.

7. Assay for glutathione peroxidase:

Glutathione peroxidase was assayed by the method as described by *Paglia* and *Valentine* [1967]. The method is based on the NADPH coupled reaction, whereby oxidized glutathione produced by the activity of GSH-PX is converted to the reduced form by exogenous GSH-R and ADPH.



The rate of GSSG formation was measured by following the decrease in absorbance of the reaction mixture in a spectrophotometer at 340 nm, due to the conversion of NADPH to NADP.

The assay mixture [3 ml] consisted of 0.05 M potassium phosphate buffer pH 7.0 , containing 0.25 mM EDTA, 2 units glutathione reductase, 2.6 mM GSH and 20 mM NADPH, to which 0.1 ml of the homogenate was added. The reaction was started by addition 1.5 mm hydroperoxide for the determination of Se dependent GSH – PX. The blank was prepared without peroxide. The solution of NADPH was prepared using its molar extinction coefficient as 6.22 x 10^3 1 mole: cm at 340 nm.One unit of the enzyme is the amount of the

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enzyme required to oxidize 1nm of NADPH/min. The activity of the enzyme is expressed as nmoles of NADPH oxidized/mg protein/ min.

8. Assay for glutathione reductase:

The measurement of the activity of glutathione reductase (GSH_R) was according o the method of *Horn* (1963).The method was a measure of the decrease in optical density due to oxidation of NADH to NADP. GSH_R is not Absolutely NADPH specific and can catalyze the reduction of oxidized glutathione by NADH but GSH-R has very low affinity for NADH.The reaction mixture (3 ml) consisted of: 2.4 ml of 0.067 M phosphate buffer pH 6.6,0.2 ml of 1.3 mM NADPH ,0.2 ml of 7.5 nM oxidized glutathione and 0.1 ml of the cytosol.The reaction was started by addition of GSSG and the decrease in absorbance at 340 nm was observed up to 3 min. Under these conditions the NADPH dependent GSSG reduction was linear with time. The change in absorbance at 340 nm should be between 0.05-0.3/min.A blank assay was performed in duplicate in two fold concentration range.The unit of enzyme is expressed as n moles of NADPH oxidized/min/mg protein.

Statistical Analyses:

We performed statistical analyses and Values are expressed as arithmetic mean and standard deviation. Experimental groups were compared with control groups using the general linear model (GLM) and pairwise t-tests for equal or unequal variances. In all analyses, p-value >0.001 was considered suggestive of an effect and p<0.001 was considered significant.

OBSERVATION AND RESULT

1.Sunscreen UV-Visible spectroscopy observation:

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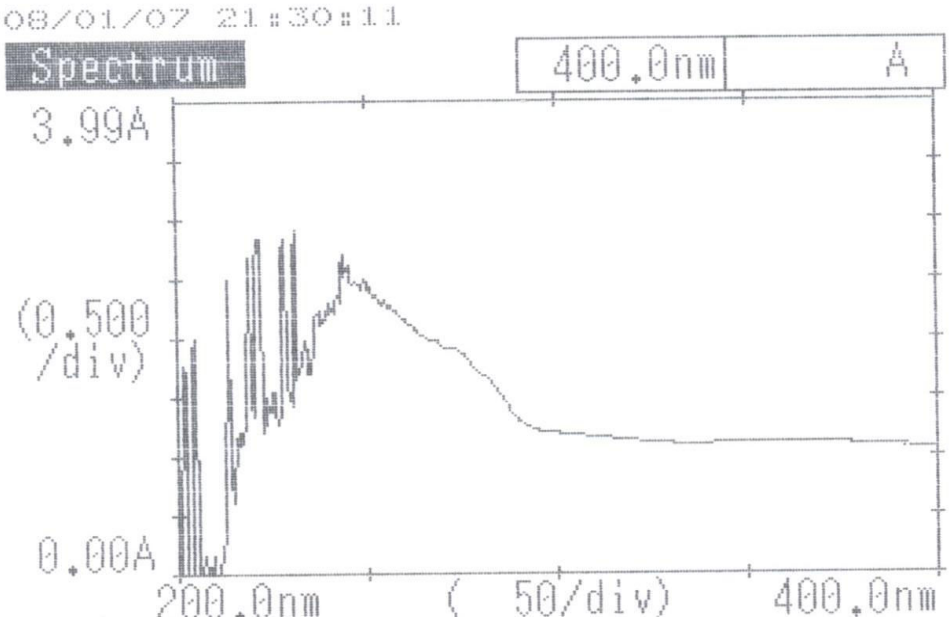

Figure-1: **Spectrum graph of Sunscreen**

The result of spectrophotometeric absorption 1.15 A is in range of 20 nm to 500 nm. Spectrum shows the max. Absorption is in the range of UV-B. The figure 1 shows the UV absorption of thin film made of Sunscreen. It is confirm that the absorption with wide range (both UVA and UVB) is observed by Nano incorporated Sunscreen. The graph 2 shows the result of spectrometric absorption of thin film of Sunscreen by UV-VIS spectrophotometer.

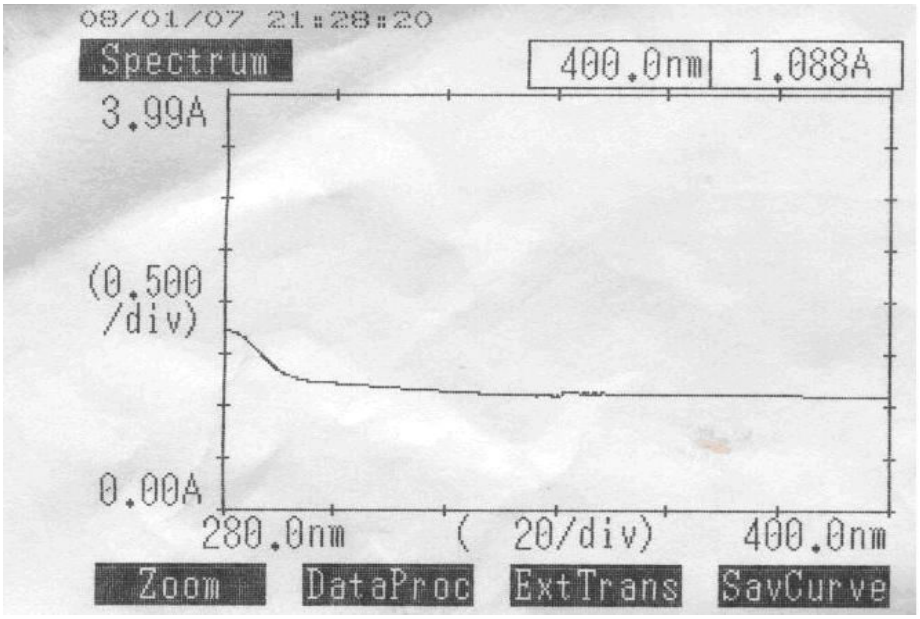

Figure-2:

Absorption spectrum by thin films of Sunscreen

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2. LDH Estimation:

In this present study lactate dehydrogenase (LDH) was estimated in six normal Albino rats and six Albino rats were treated with UV and six Albino rats were treated with protective action of sunscreen by UV radiation. LDH estimation was done by colorimetric method devised by *king-wooton* (1964). Total serum LDH activity was observed in UV treated Albino rats evaluate significantly higher in group a and b, 2842 ± 3.6, 1854 □ 1.0 as compare to control 1729 ± 2.0. UV treated with protective action of sunscreen shows significant decline result in group a' and b' 2779.371.28, 1750 ± 5.00 respectively. The graphs represent the less activity of LDH after application of Nano ZnO sunscreen.

Table–1: **Lactate dehydrogenase (LDH) activity in the UV-irradiated Albino rats.**

Group	**Serum U/L**		
	Lowest	**Highest**	**Mean ± S.D.**
Group-a	2842	2845	2842 ¯ 3.6**
Group-a'	2700	2800	2779.3 ¯ 71.2**
Group-b	1853	1855	1854 ¯ 1.00**
Group-b'	1745	1755	1750 ¯ 5.00*
Control	1727	1731	1729 ¯ 2.0

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

LDH Activity U/L

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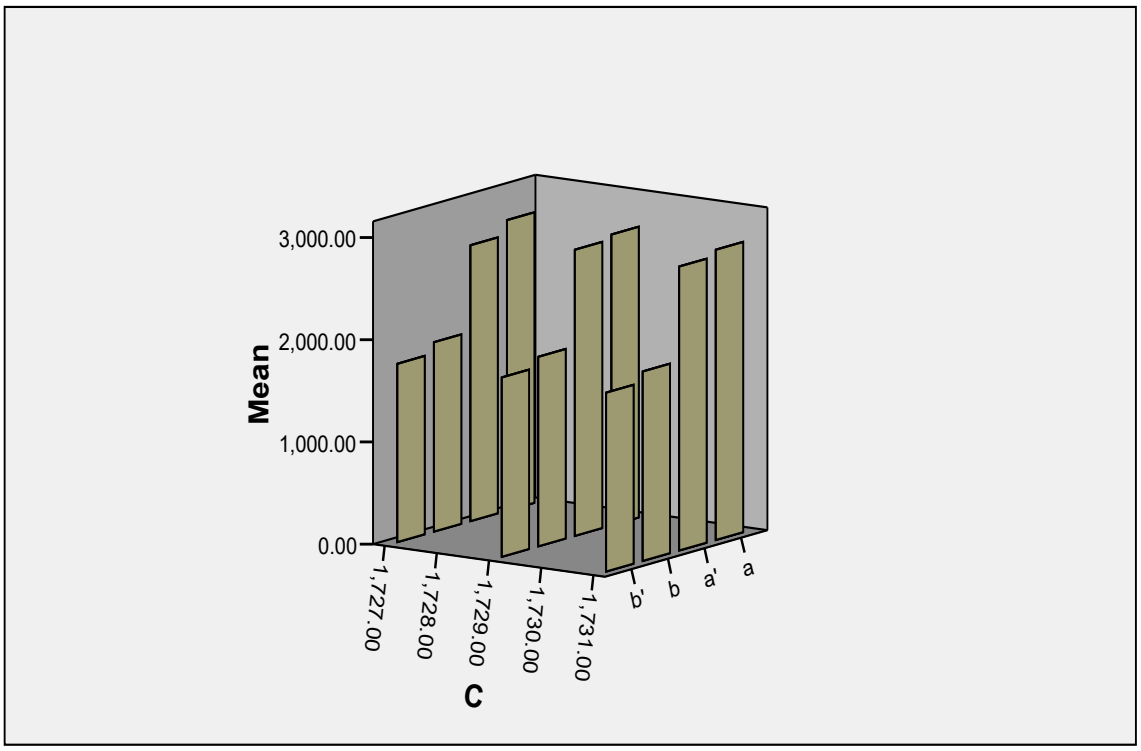

Figure-3: **Graph of LDH Activity in Serum**

3. Alkaline Phosphatase:

Alkaline phosphatase was produced primarily in the liver and in bone. High ALP levels can be caused by bone diseases, bone tumor, kidney cancer. Alkaline phosphatase catalyzes the hydrolysis of P-nitrophenol and phosphate in alkaline medium.

P-nitrophenyl phosphate + H_2O □ P-nitrophenol + H_3PO_4

Alkaline phosphatase (ALP) activity in sample was determined by measuring the per unit time absorption increased at 405nm. Total serum alkaline phosphatase was significantly increased in UV-irradiated Albino rats a and b, 150.35 $\pm$ 0.56, 102 $\pm$ 1.00 respectively compare to control 27.34 $\pm$ 0.01. Level of alkaline phosphatase in Albino rats protected with sunscreen (nano ZnO) in a' and b' was observed significantly lower 147.33 $\pm$ 2.51, 93.66, $\pm$ 7.56 respectively. This estimation reveals protective action of Nano ZnO to that correspondence less antioxidant produced against UV.The Graph is representing the systemic effects of UV and Protection action of Nano ZnO Sunscreen.

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Table-2: **Serum Alkaline Phosphatase (ALP) enzyme activity in the UV-irradiated Albino rats**.

Group	**Serum U/L**		
	Lowest	**Highest**	**Mean ± S.D.**
Group-a	150.1	151.00	150.35 ¯ 0.56**
Group-a'	145	150	147.33 ¯ 1.45**
Group-b	101	103	102 ¯ 0.57**
Group-b'	89.64	101.68	94.66 ¯ 4.37**
Control	27.32	27.35	27.34 ¯ 0.01

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

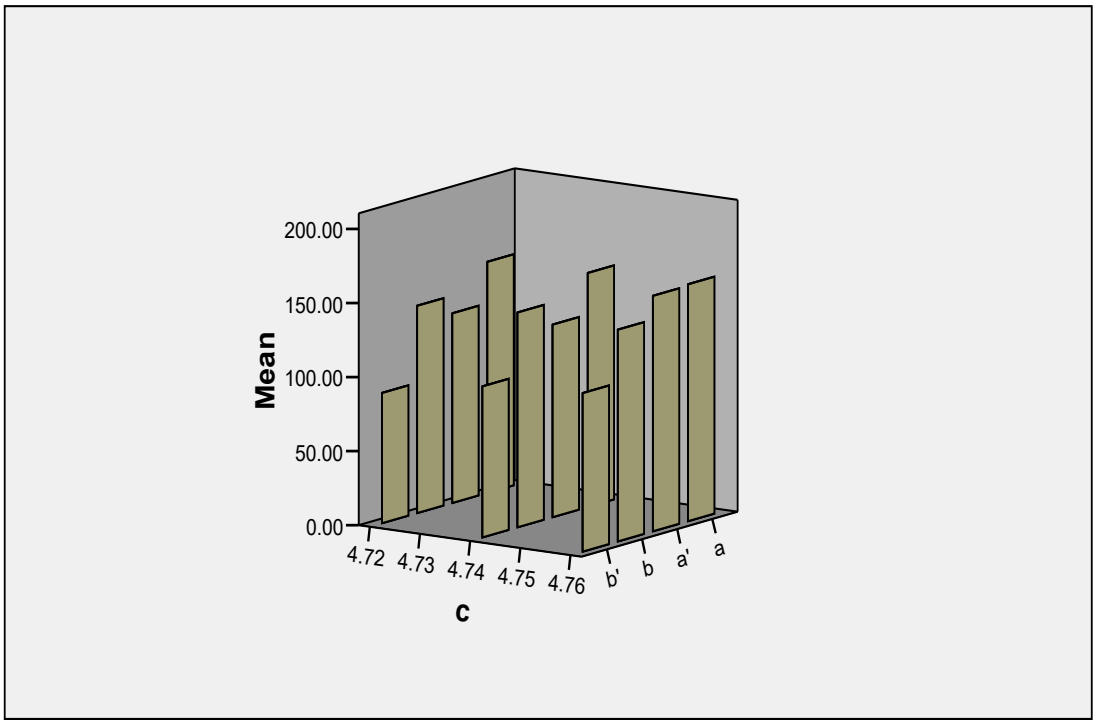

Figure-4: **Graph of ALP activity in Serum**

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4. Acid Phosphatase:

The enzymatic reaction sequence employed in the as a of acid phosphatase is as follows:

□- Naphtylphosphate + H_2O □ □-Naphthol + inorg-phosphate

□-Napthol + Fast Red TR □ □-Diazo Dye (Chromophore)

The □-Napthol release from the substrate □- Naphtylphosphate by acid phosphatase is coupled with Fast Red TR to produce a colored complex which absorbs light at 405nm. The reaction can be quantitated photo metrically because the coupling reaction i.e. instaneous. In present study values of acid phosphatase observed in group a and b were significantly increase 158 ± 2.00, 142.67 □ 2.52 respectively and in a'and b' were observed significantly decline 139 □ 17.33, 99± 9.8 respectively with control value 4.74 ± 0.02.The graph representing the effects of UV on AP and protective action of sunscreen .

Table-3: **The Acid Phosphatase activity in the UV-irradiated Albino rats**.

Group	**Serum U/L**		
	Lowest	**Highest**	**Mean ± S.D.**
Group-a	156	160	158 ¯ 1.54**
Group-a'	128	159	139 ¯ 17.34**
Group-b	140	143	142.67 ¯ 2.52*
Group-b'	88	107	99 ¯ 9.8**
Control	4.72	4.76	4.74 □ 0.02

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

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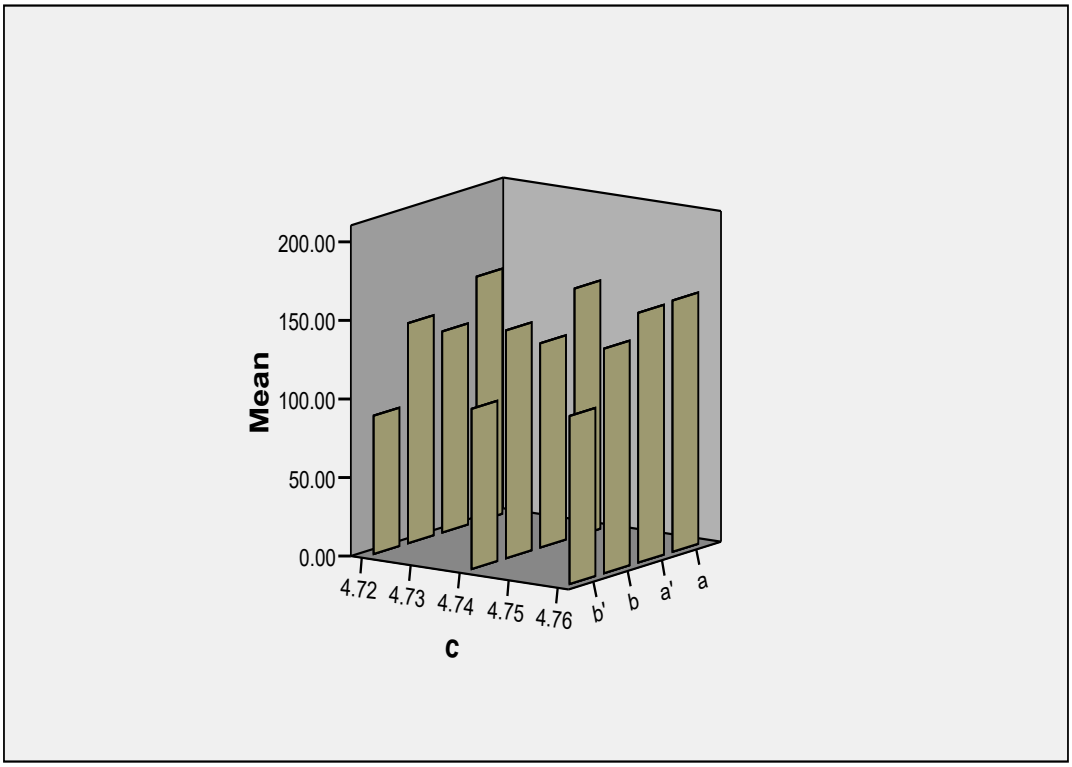

Figure-5**: Graph of AP activity in Serum**

5. Total Protein Assay:

Total protein level in group a is 3.28 ± 1.047 gm/L and in b is 4.98 ± 2.6gm/L, significantly lower as compared to sunscreen protected groups a' and b' shows significant higher level 4.67 ± 0.28 gm/L, 5.24 ± 0.036gm/L respectively, with control 6.87 ± 0.90gm/L.

Table–5: **Total Serum Protein in UV-irradiated Albino rats**.

Group	**Serum Gm/L**		
	Lowest	**Highest**	**Mean ± S.D.**
Group-a	2.19	4.28	3.28 □ 1.04**
Group- a'	4.5	5.00	4.67 □ 0.28*
Group- b	2.28	5.00	4.98 □ 2.69*
Group-b'	5.25	5.27	5.24 □ 0.036*
Control	6.00	7.81	6.87 □ 0.90

1) Group-a = UV treated for 45 sec.-1 min.,

2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,

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3) Group-b = UV treated for 15 sec ,

4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

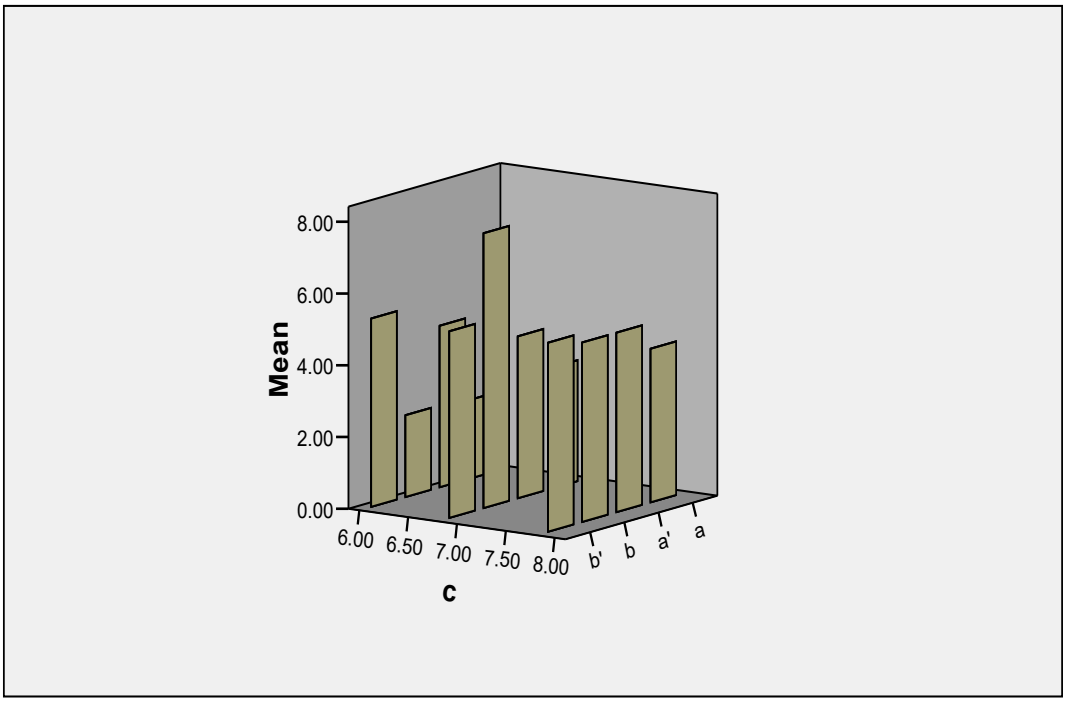

Figure-6: **Graph of Total Protein Concentration in Serum**

6. Antioxidant assay in skin:

In thesis enzyme activities expressed as units/gram of skin and units/milligram of protein .When the data are expressed as units/milligram of protein, alloenzyme activities are the same in dermis and epidermis, except for SOD, which was higher in dermis than in epidermis (by 100%). When the values are expressed in units/gram of skin than GR were higher in epidermis than in dermis by 49%, 86% and 74% respectively.

7. SOD after Irradiation:

SOD activities in epidermis and dermis were not different when expressed as units/gram of skin. Values observed for group a and group b are 368.8±16.4,612.6± 7.0, are significantly lower in a group and more effective in b group with compare to control and UV protectant group. The value of SOD value observed for epidermis protein is significant lower 11.7 ± 1.4

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U/mg protein, for dermis control group calculated value is 27.5 ± 2.5 U/mg protein. The Graph is presenting the overview of activity of SOD in Rat.

Table–7: **SOD Activities in Epidermis and Dermis of the Albino Rat**

Group	Epidermis U/g Skin			Dermis U/g
	Lowest	Highest	Mean ± S.D.	Mean ± S.D.
Group-a	350	380	368.8±16.4**	600 ± 7.48**
Group-a'	470	510	479 ± 9.5**	640±6.68**
Group-b	606	620	612.6± 7.0*	840±6.48
Group-b'	680	700	690±10.0*	860±4.08**
Control	700	712	707±6.4	900±12.24

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

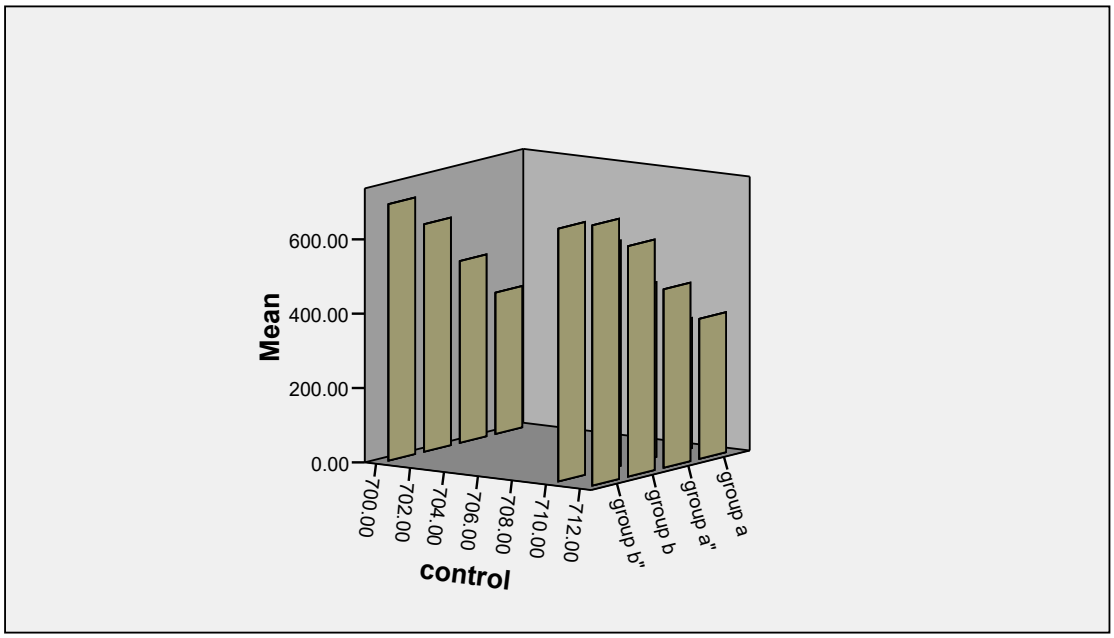

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Figure-7**: Graph of SOD activity in skin**

8.Glutathione estimation after Irradiation:

The Value observed for group a and group b are significantly lower 680± 9.1, 720±25.00 respectively with compare to control and UV protect ant group a' and b' are 752 ± 10.44, 692±12.00 and 740± 16.7 in epidermis. The value observed for Dermis are significantly lower in 200± 2.3, 270 ± 4.08 for a and b groups. The values for control and UV protectant group are320± 3.26 and a' 210± 8.60 and b' 280±8.04.

Table-8: **GSH Activities in Epidermis and dermis of the treated group**

Group	n mol/g of Epidermis			Dermis
	Lowest	Highest	Mean ± S.D	Mean ± S.D
Group-a	670	682	680± 9.1*	200± 2.3*
Group-a'	680	704	692±12.00**	210± 8.60**
Group-b	695	745	720±25.00**	270± 4.08**
Group-b'	722	743	740± 16.7**	280±8.04**
Control	740	759	752 ± 10.44	320± 3.26

1) Group-a = UV treated for 45 sec.-1 min.,

2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,

3) Group-b = UV treated for 15 sec ,

4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

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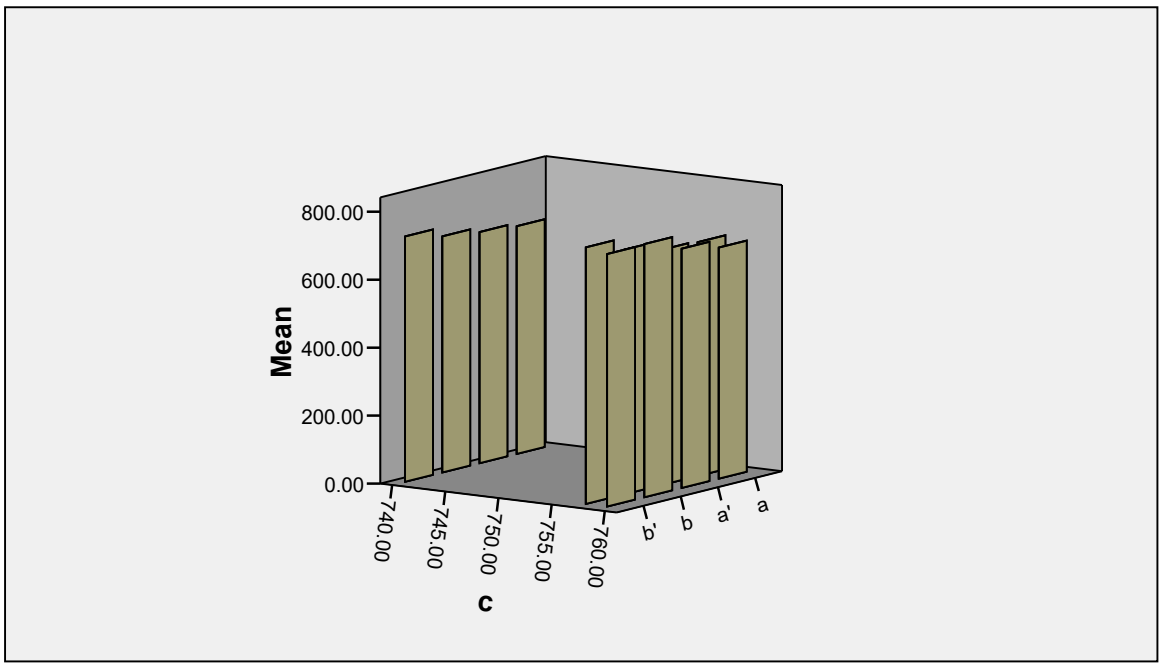

Figure-8**: Graph of GSH activity in skin**

9. Glutathione peroxidase estimation after Irradiation :

The Value observed for group a and group b are significantly lower 1.46±0. , 1.56± 0.06respectively with compare to control and UV protectant group a' and b' are a'1.98+0.02and b'2.16+0.06 in epidermis. The value observed for Dermis are significantly lower in group a 0.90±0.045 and group b1.10± 0.48 groups. The values for control and UV protectant group are 1.32± 0.06 and a'1.06± 0.069 and b'0.32± 0.032. The value of GPx value observed for epidermis protein is 0 .049 ± 0.003 U/mg protein for control group was calculated and for dermis is0.040 ± 0.004 U/mg protein.

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Table-9: **Glutathione Peroxidase Activities in Epidermis and dermis of the treated group**

Group	Epidermis U/g Skin			Dermis U/g Skin
	Lowest	**Highest**	**Mean ± S.D.**	**Mean ± S.D.**
Group-a	1.45	1.47	1.46+0.01*	0.90+ 0.045*
Group-a'	1.96	2	*1.98*+0.02**	1.06+ 0.069**
Group-b	1.50	1.62	1.56+ 0.06**	1.10+ 0.48**
Group-b'	2.10	2.22	2.16+0.06*	0.32+ 0.032*
Control	2.37	2.55	2.46+ 0.09	1.32+ 0.06

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

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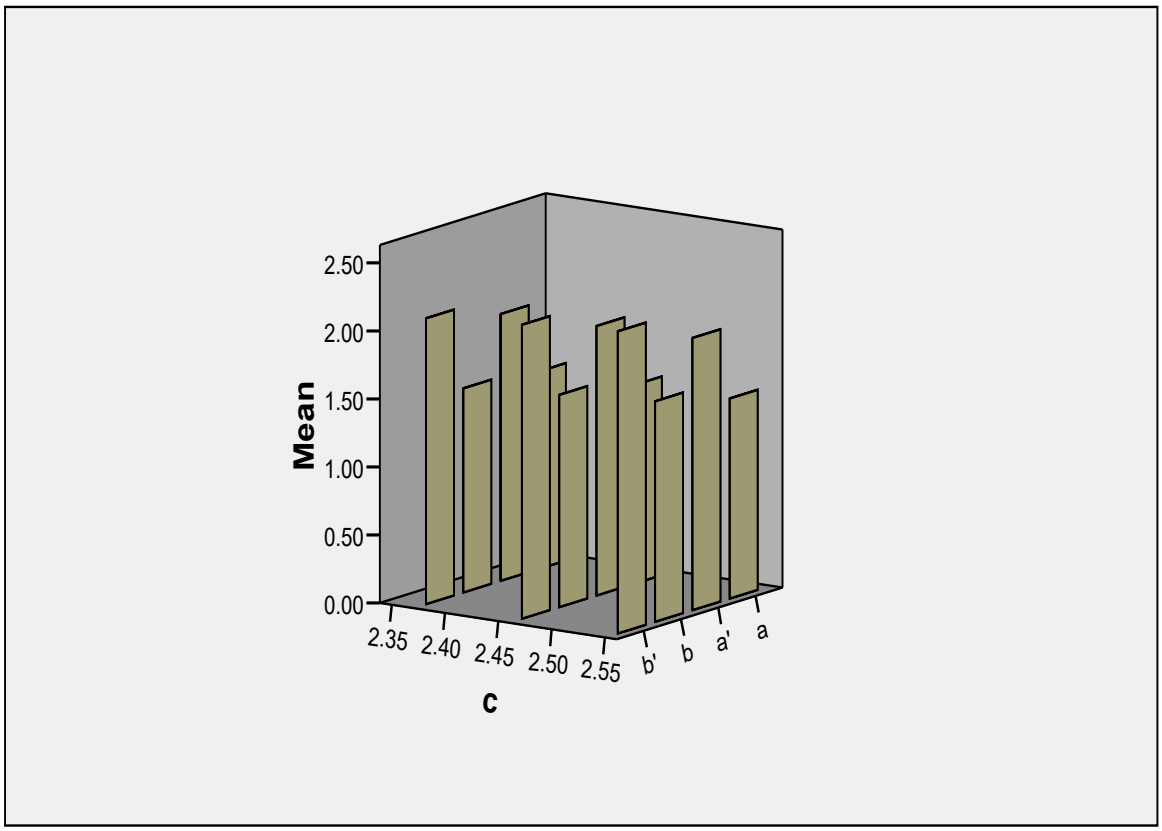

Figure-9**: Graph of GPx activity in Skin**

10. Glutathione Reductase estimation after Irradiation:

The Value observed for group a and group b are significantly lower 0.90± 0.035 , 2.10±0.0244respectively with compare to control and UV protect ant group a' and b' are2.42±0.02 ,2.10±0.08 and 2.20±0.78 in epidermis. The value observed for Dermis are significantly lower in 0.56± 0.03, 1.10±0.04for a and b groups. The values for control and UV protectant group are1.39±0.09and a'1.00± 0.1 and b'1.32±0.05. The value of GR value observed for epidermis protein is0.042 ± 0.001 U/mg proteins for control group was calculated and for dermis is 0.041 ± 0.001 U/mg protein.

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Table–10 : **Glutathione Reductase Activities in Epidermis and Dermis of the treated group**

Group	Epidermis U/Gm			Dermis U/Gm
	Lowest	Highest	Mean ± S.D.	Mean ± S.D.
Group-a	0.85	0.93	0.90± 0.035**	0.56± 0.03**
Group-a'	2.03	2.22	2.10±0.08**	1.00± 0.1**
Group-b	2.07	2.13	2.10±0.0244**	1.10±0.04**
Group-b'	2.11	2.30	2.20±0.78*	1.32±0.05**
Control	2.40	2.45	2.42±0.02	1.39±0.09*

1) Group-a = UV treated for 45 sec.-1 min.,
2) Group- a' = UV treated with protective action of sunscreen for 45 sec.-1 min.,
3) Group-b = UV treated for 15 sec ,
4) Group-b' = UV treated protective action of sunscreen for 15 sec.5)control value. p<0.001.....**, p>0.001.....*.

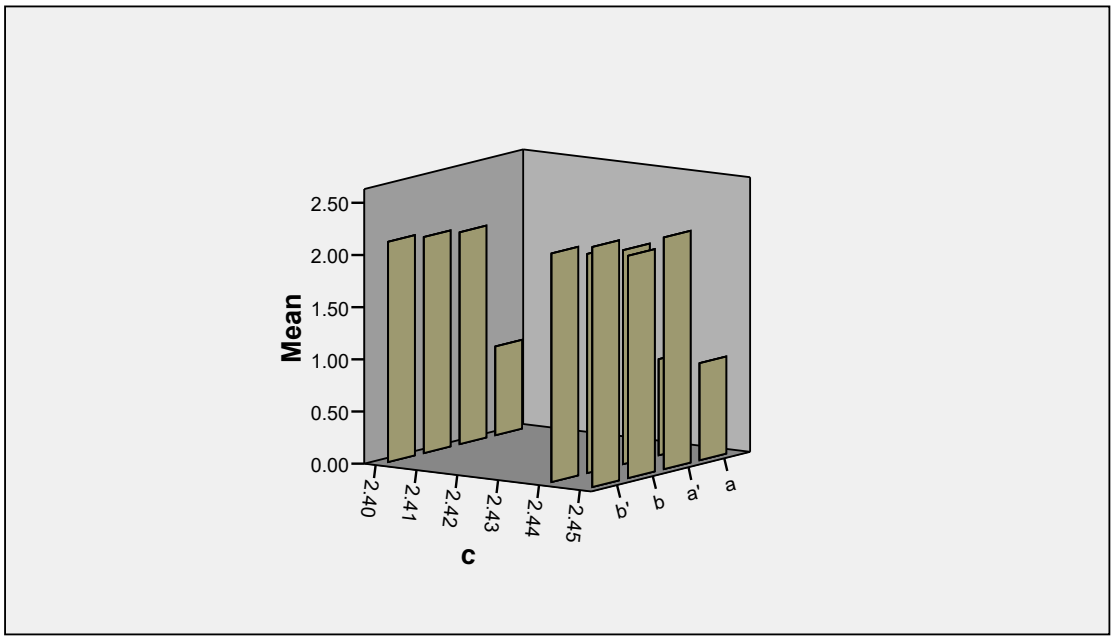

Figure-10**: Graph of GR activity in skin**

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The change in activities of antioxidant enzymes in the irradiated side compared with the protective covering of Sunscreen and control group is maintained. Activities of glutathione peroxidase and GR in both epidermis and dermis decreased slightly but significantly (at least $p < 0.001$ for all). SOD activities exhibited large and significant decreases with irradiation (36% and 48% for SOD; at least $p<0.001$ for all). The concentration of GSH and total glutathione decreased with irradiation in both epidermis ($p < 0.001$for both) and dermis ($p < 0.001$ for total).

Studying the UV absorption by Nano ZnO Sunscreen by the part of skin that is directly exposed to UV light is of considerable interest because UV-induced damage to such defense provides indirect evidence for free-radical processes because the exact nature of such damage may help elucidate mechanisms of free-radical damage and because rational preventive strategies can only be designed if it is known which antioxidants are most depleted by UV radiation. In this study, it is presented the comprehensive analysis of antioxidants, redox status, and effects of in vivo UV irradiation in epidermis and dermis and action of ZnO for protection as UV shielding property.

In thesis enzyme activities expressed as both units/gram of skin and units/milligram of protein and non-enzymic antioxidants as nanomoles/gram of skin. Traditionally, enzyme activities have been expressed as units/milligram of protein to determine degree to purity during separation and purification, but there is no a proper reason for referencing activity to protein. It is known that values expressed as units or nanomoles/gram of skin are more reasonable than those expressed per milligram of protein because much collagen in dermis is not extracted by the usual homogenization method, so that values expressed per milligram of protein are relatively higher, compared with epidermis, than those expressed per gram of skin, however, because collagen is involved in photo damage to dermis (e.g. photo aging) (*Smith* et.al.,1962; *Lavker* et al.,1987), concentrations of antioxidants, which may help prevent such damage expressed in a form that accounts for collagen; that is, per gram of skin. All intracellular and extra cellular materials are potentially subject to free-radical attack. For some pathologies (e.g., cancer), the final target may be DNA, but other targets, such as lipids, may serve is important intermediaries in DNA damage. For other pathologies (e.g. cutaneous photo aging), multiple targets, including but not limited to cell proteins, may be involved. Therefore, the reference base selected should reflect all cellular components. Wet weight reflects all cellular components, whereas protein is only one component**.**

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DISCUSSION

ZnO nanocrystals of Wurtzite structure have been synthesized using a modified sol-gel procedure. The small crystallite size and high surface area allows the nano ZnO to exhibit a unique surface chemistry.XRD results shows that the synthesized ZnO nanoparticles is of hexagonal wurtzite structure with very good crystallinity. XRD studies were carried out on a *Philips analytical diffractometer*. The light source used was CuK_{α} radiation with applied voltage of 40 kV .2θ angles ranged from 1.5^0 to 10^0 at a scan rate of 0.2^0 min^{-1} and at a step size of 0.02^0 with a view to monitor the structural changes in the host matrix as a result of ZnO loading. All peaks in the pattern match well with the characteristic reflections of ZnO reported by *Faal Hamedani*(2006) and confirm the phase purity of the samples.

Nano sized ZnO shows 100% absorption in range of UV, confirm that it acts as a good UV filter in sunscreen. The method is accordance with the work done on reflection by *Dodd*(2006).

Nano ZnO is a lipophilic endogenous antioxidant that provides protection against UV induced oxidative membrane damage. As per the conclusion of SCCNFP there are more inflammation is needed to enable a proper safety evaluation of Nano ZnO for us as UV filters in cosmetic products (*Ryan* et al., 2000). In this research it is observed that sunscreen with nano ZnO shows protective action against UV radiation without any irritability and sensitivity. The estimation of LDH, ALP, and Acid Phosphatase in serum is an objective diagnostic adjunet in the diagnosis of photocarcinogenesis and photo aging damage. ZnO is suitable for the using sunscreen products to block the UV. The main concern of the present evaluation is related to the assessment of Nano ZnO (20-500 nm), which may be coated by other compounds and is used as an important ingredient in sunscreen formulations.

It is widely hypothesized that UV irradiation induces free-radical formation in skin. This has been shown to occur in isolated whole skin (*Norrins* et al.,1962; *Pathak* et al.,1962) and skin homogenates (*Nishi* et al.1991; *Ogura* et al., 1991), but technical difficulties make demonstration of UV-induced free-radical formation *in vivo* extremely difficult. If UV light or other environmental factors cause cutaneous free-radical formation *in vivo*, the epidermis, being the outermost layer of the skin, would be expected to have the greatest antioxidant defenses. SOD, is 50-85% higher in epidermis when expressed as units/gram of skin. The results of this study are in accordance with the results of *Connor and Wheeler* (Connor *et*

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al.,1987) for glutathione and related enzymes in skin from Albino rats of the same strain; they reported glutathione concentrations of 750 nmol/g skin in epidermis (3% oxidized) and 320nmol/g skin in dermis (22% oxidized);here in thesis found similar percentages for the oxidized form in the epidermis and dermis (5.5% and 21.7%, respectively), although our absolute values were approximately 50% higher. This may be due to differences in epidermal separation methods because these scientists immersed the samples in water, thus increasing the weight due to water absorption. Whereas in this study the samples were not exposed directly to water. Results of this study for relative activities of GR and glutathione peroxidase in epidermis and dermis are 74% and 86% greater in epidermis when expressed per gram of tissue, these results are also in accordance with those of Connor *and Wheeler,* they reported approximately twice the activities of these enzymes in epidermis as compared to dermis, when expressed per gram of tissue (*Connor* et al., 1987). The results of SOD differs with those of *Kim and Lee 1987* for human skin when expressed as units/milligram of protein , it show dermis to have over twice the activity of epidermis, whereas this works report 20% greater activity in epidermis. This may represent a difference between human skin and rat skin. These investigators did not report their findings as units/gram of skin. The fact that these values as well as those for the other epidermal free-radical formation, necessitating greater antioxidant capacity.

Glutathione is a thiol-containing tripeptide which, in its reduced state, participates in several functions of vital importance to the cell. It maintains in the proper redox state the thiol groups of soluble and structural proteins and participates in the detoxification of hydroxyperoxides and □-oxoaldehydes (*Flohe* et al, 1976). Reduced glutathione also detoxifies a variety of potentially harmful electrophilic compounds which are later excreted as mercapturic acids (*Chasseaud* 1976). Glutathione is reduced from its oxidized disulfide form by glutathione reductase, which employs NADPH generated by the hexose monophosphate pathway (*Meister* 1975). The result of spectrophotometric absorption 1.15 A was in range of 200 nm to 500 nm .Spectrum showing the max. Absorption in range of UVB. The UV absorption by thin film made of Sunscreen (Nano ZnO), confirmed the absorption with wide range (both UVA and UVB). Activities of glutathione peroxidase and GR, SOD, GSH in both epidermis and dermis were significantly observed. The change in the activity of ALP, total Protein,LDH,AP, were observed in the serum which shows the effect of UV and Nano sunscreen protective action.

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