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"BIOCHEMICAL STUDIES ON ANTIOXIDANT PROPERTY OF *WITHANIA SOMNIFERA* (ASHWAGANDHA) IN HUMAN ERYTHROCYTES"

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ABSTRACT

Oxidative stress, driven by an excess of reactive oxygen species (ROS), is a key contributor to cellular damage and the development of numerous diseases. This study evaluates the antioxidant potential of *Withania Somnifera* (Ashwagandha) root extract in protecting human erythrocytes from oxidative damage. An aqueous-alcoholic extract was tested in vitro through multiple biochemical assays, including hydrogen peroxide and hydroxyl radical scavenging, lipid peroxidation (MDA), and osmotic fragility. Results showed that *Withania Somnifera* significantly reduced oxidative stress markers, enhanced cellular antioxidant defense, and stabilized erythrocyte membranes. These findings support its role as a natural antioxidant with potential therapeutic applications in managing oxidative stress-related conditions.

Keywords: *Withania Somnifera,* Erythrocytes, Reactive oxygen species

I. INTRODUCTION

Over the past century, the major health threats faced by humanity have evolved dramatically, while infectious agents were the primary cause of mortality among our ancestors, Today's challenges are increasingly linked to modern lifestyle choices and environmental exposures. In the present era synthetic chemicals, toxic heavy metals, environmental pollution, and psychological stress have emerged as significant contributors to various chronic illnesses. Although oxygen is essential for life, its paradoxical nature also plays a central role in biological damage. Oxidative stress imbalance between ROS production and antioxidant defense system is now recognized as a leading cause of cellular and tissue damage. Free radicals are chemically unstable molecules, that possess unpaired electrons which makes them highly reactive. They can be formed endogenously through normal metabolic activities such as mitochondrial transport, enzyme reaction, and immune responses or exogenously through exposure to radiation, tobacco, smoke, pollution, and other environmental toxins. These reactive species include superoxide anions (0_2), hydrogen peroxide (H_2O_2) hydroxyl radicals (OH), and hydroperoxyl radicals (HO_2) which can interact with vital biomolecules such as DNA, proteins, lipids, and carbohydrates, ultimately disrupting cellular function. The Plasma membrane is crucial for maintaining cellular deformability and survival. However oxidative stress can compromise membrane stability resulting in osmatic fragility & hemolysis. (Halliwell B, Gutteridge JMC & Cross CE et. al, 1992)

Exogenous antioxidants through diet or therapeutic supplementation have become increasingly important: Natural antioxidants – Primarily serviced from fruits, vegetables, and medicinal plants, are especially valued for their efficacy and safety. Natural antioxidants include vitamins such as a-tocopherol (vitamin E), vitamin A, vitamin C and a variety of Polyphenolic compound unlike synthetic oxidants such as butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), and dimethyl sulfoxide (DMSO). W.S. (ashwagandha) has been extensively studied for its adaptogenic, anti-inflammatory, and anti-oxidative properties. Its bioactive phytochemical components withanolides & alkaloids have demonstrated efficacy in scavenging free radicals (Lakshmi C.M. et. al, 2000).

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In the present study, we have explained the antioxidant potential of W.S. using an aqueous-alcoholic extract (60% alcohol & 50% DW). The Investigation focused on its effect on human RBC (erythrocytes) under in-vitro conditions.
Botanical name: *Withania Somnifera* (Dunal)
Family: Solanaceae (Nightshade family)
Habit: Shrub
Cultivation: *Withania Somnifera* grows well in sandy loam or light red soil with good drainage. The semi-tropical areas receiving 500-750 mm rainfall are suitable for cultivation of this rained crop. The crop requires a dry season during its growing period. Temperature between 20ºC to 35ºC is most suitable for cultivation. Late winter rains are conducive to the proper development of the plant roots.

II. MATERIAL AND METHOD

Washing: All the glassware used during the experiments was first thoroughly washed with detergent and then with chromic acid followed by prolonged drench in tap water and finally rinsed with the double distilled water. This washed glassware was then dried in a hot air oven.
Measurement of pH: All pH measurements were performed on a pH meter. The instrument was regularly calibrated with the help of standard buffer solutions (pH 4.0, 7.0, and 9.2) prepared from standard buffer tablets.
Optical measurements: All routine colorimetric estimations were performed on the Shimadzu double-beam spectrophotometer.
Isolation of erythrocytes: The blood sample obtained was centrifuged at 4oC for 10 minutes at 3500 rpm in a Remi C24 refrigerated centrifuge machine using 15 ml angle head rotor. Plasma and buffy coats were removed. Care was taken to eliminate Leukocyte contamination. The isolated erythrocytes were washed 4 to 5 times with 0.154 M NaCl (isotonic saline) to obtain completely washed pure Erythrocytes free of leukocyte contamination.

Preparation of extract:

Withania Somnifera extract was prepared in soxhlet assembly. Aqueous-alcoholic extract was prepared by using 50% distilled water and 50% alcohol. The soxhlet was run for 72 hours at a temperature between 60ºC to 80ºC.
Soxhlet: Soxhlet is a small-scale extraction apparatus consisting of a flask, a Soxhlet extractor, and a reflux condenser. The raw material (powder of Withania Somnifera 10 gm is placed in a thimble made of cotton and inserted into a wide central tube of the extractor. The solvent (50% alcohol and 50% distilled water) is placed in the flask and boiled, its vapors passing up the right-hand tube into the central space above the powder and then to the condenser. The condensate then drops back onto the powder, through which it percolates, leaching of solute in the process. When sufficient solution has been collected to raise its level to that of the lap of the siphon tube, the whole of the collected percolates siphons over into the flask. The suction effect of the siphons assists the permeation of solvent through the drug. A limited amount of hot salient assists is thus made to percolate repeatedly through the new material the solute from which is transferred to the flask. The principle of continuous hot extraction is sometimes used to extract a drug for assay. It is the simple form of the apparatus which has the advantage that the hot, rising vapors; encircle the

material to be extracted. Finally, the solution was dried in a vacuum. The extract was dissolved in DMSO (1mg Withania Somnifera extract/ml of DMSO).

1. Hydrogen Peroxide (H_2O_2): -

Reactions that generate hydrogen peroxide by shifting two electrons to oxygen have been known for some time. Although toxic, its ability to form the hydroxyl radical renders it extremely dangerous. The superoxide radical reacts with hydrogen peroxide, in the Haber-Weiss reaction, to form the extremely reactive hydroxyl radical (HO·). (Fridovich I, 1976)

$$O_2^{-}\cdot + H_2O_2 \longrightarrow HO\cdot + HO\cdot + O_2$$

Reagents-

Phosphate buffer- 50mM (pH-7): Dissolved (a) 1.70 g KH_2PO_4 and (b) 2.22 g $Na_2HPO_4.2H_2O$ in D.W, volume maintained 250 ml each, Mix solution (a) and (b) in proportion 1:1.5 (v/v).

H_2O_2- 30mM: 0.34 ml 30% H_2O_2 was diluted with phosphate buffer to maintain volume up to 100 ml.

Procedure-

The reaction mixture contains different concentrations of Withania Somnifera extract (1-10,000 µg/ml of DMSO) and the volume was maintained to 2 ml by adding phosphate buffer. The reaction was started by adding freshly prepared 1 ml H_2O_2 to each tube containing the reaction mixture. Mixed well and followed the decrease in absorbance at different time intervals (0 sec, 30 sec, 1 min, and 2 min, etc.). Absorbance was measured spectrophotometrically at 240 nm.

2. Hydroxyl (OH) Radical: -

Hydroxyl radicals were generated by the Fe^{3+}-ascorbate-H_2O_2 system (Rowley and Halliwell, 1983). The formation of hydroxyl radicals appears to occur by the following reaction:

$$Fe^{3+}\text{- EDTA} + AH^{-} \longrightarrow Fe^{2+}\text{- EDTA} + A^{-}$$

$$Fe^{2+}\text{- EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{- EDTA} + OH + OH^{-}$$

$$AH + H_2O_2 \longrightarrow O_2 + OH + OH^{-}$$

Hydroxyl radicals (OH) so formed were quantitated by their ability to attack aromatic compounds with the formation of hydroxylated products. In this study

salicylate (2-hydroxy benzene) at a concentration of 2.5 mM is included in the reaction mixture, and the hydroxylated product (2, 3-dihydroxy benzoate) is extracted with ether and assayed spectrophotometrically at 510 nm.

Reagents- 2.5 mM salicylate, 0.3 mM EDTA, 0.1 mM $FeCl_3$ (fresh), 2mM ascorbate (fresh), 150 mM KH_2PO_4-KOH buffer (pH-7.4), 1 mM H_2O_2, 11.6 N HCl, 0.5g NaCl (in each tube), chilled Diethyl ether, cold double D.W, 10 % TCA, 0.5M HCl, 10 % sodium tungstate, 0.5 % sodium nitrite (fresh) and 0.5 M KOH.

Method-

The reaction mixture of 2 ml contains 2.5 mM salicylate, 0.3 mM EDTA, 0.1 mM $FeCl_3$ (fresh solution just before use), 2 mM ascorbate (fresh), 150 mM KH_2PO_4-KOH buffer (pH 7.4), and 10 µl Withania Somnifera extract of different concentration (1-10,000 µg/ml of DMSO), reaction was started by the addition of 1 mM H_2O_2. The reaction mixture was incubated for 90 minutes at 37ºC. At the end of incubation, the reaction was stopped by adding 80 µl (11.6 N) HCl and 0.5g NaCl.

The hydroxylated product (2, 3-dihydroxy benzoate) was then extracted with 4 ml chilled diethyl ether. 3 ml of the upper layer was taken and evaporated to dryness on the sand bath. The residue so obtained was dissolved in 0.5 ml cold double distilled water. To these reagents were added in order 0.25 ml 10 % (w/v) TCA dissolved in 0.5M HCl, 0.5 ml 10 % (w/v) sodium tungstate, and 0.5 ml 0.5 % (w/v) sodium

nitrite (freshly prepared). Stand the reaction mixture at room temperature for 5 minutes and then add 1 ml 0.5M KOH and read the absorbance at 510 nm.

Calculation-
0.651 nm = 200 n mole of hydroxylated product (2, 3-dihydroxy benzoate).

3. Determination of red blood cells osmotic fragility:
Experiment with Withania Somnifera extract:
In vitro experiments were carried out by dissolving Withania Somnifera extract in DMSO to get the appropriate concentration of 1-10,000 µg/ml of DMSO. The whole blood was incubated at 37°C for 30 minutes. Parallel control experiments were performed in which blood was incubated without Withania Somnifera extract containing an equal amount of DMSO.

Reagents-
1. Heparinized blood
2. Stock PBS (equivalent to 10 % NaCl)
3. Hypotonic PBS (equivalent to 0.10 % to 0.85 % NaCl by diluting stock PBS)

Method-
Osmotic fragility (OF) experiments were performed by preparing hypotonic phosphate buffer solutions (PBS) equivalent to 0.10 % to 0.85 % NaCl by diluting stock phosphate buffer saline. Phosphate buffer saline was prepared and the experiment was performed according to method of Dacie and Lewis (1984) extent of hemolysis was measured calorimetrically at 540 nm with a yellow-green filter. Stock phosphate buffer saline was made by dissolving 25 gm NaCl, 6.825 gm Na_2HPO_4 and 1.215 gm $NaH_2PO_4.2H_2O$ in 500 ml distilled water to make a solution osmotically equivalent to 10 % NaCl which was diluted with distilled water to make a hypotonic solution of varying concentration. Heparinized blood was added to a hypotonic solution of different concentrations of NaCl (PBS) in the proportion of 1 to 100. Generally, 0.05 ml of the blood was added to 5.0 ml of the phosphate buffer saline and mixed thoroughly. After keeping it at room temperature for 25 minutes the hypotonic solutions were centrifuged for 5 minutes at 3500 rpm in a clinical centrifuge. The optical density of the supernatant was determined at 540 nm by using a supernatant of 0.85 % NaCl (PBS) hypotonic solution in which undetectable lysis took place as blank. The supernatant of 0.1 % NaCl (PBS) hypotonic solution extent maximum lysis took place was arbitrarily delimited to have shown 100 % lysis. The extent of hemolysis was calculated by dividing the absorbance of varying hypotonic solutions by the absorbance of the solution showing maximum hemolysis [0.1 % NaCl (PBS)].

$$\text{Extent of hemolysis} = \frac{\text{OD of supernatant of varying NaCl \%}}{\text{OD of supernatant with maximum lysis (0.1 \% NaCl)}} \times 100$$

The erythrocyte hemolysis curve was prepared by plotting the percent of NaCl solution on the X-axis and the percent hemolysis on the Y-axis. The osmolyte curve represents the cumulative frequency of the individual erythrocyte fragility present in the blood under study (Detraglia 1974) and the osmolarity curve, the percent of NaCl (PBS) in which 50 % hemolysis takes place represents the fragility of the maximum numbers of the cell, which is expressed as mean erythrocyte fragility (MEF).

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4. Lipid peroxidation (MDA):

MDA is the most abundant individual aldehyde resulting from lipid peroxidation and its determination by TBA is the most common method of estimating lipid peroxidation. (Esterbauer H and Cheeseman KH, 1990)

Reagents-

Krebs ringer phosphate buffer (KRP) (pH-7.4)
(KRP- with 5mM glucose (KRPG) (pH-7.4), 10 % TCA, 0.05 M/l of NaOH, 0.6 % TBA,
MDA

Method-

- Washing of blood to get packed RBC-
- Washed blood 2-3 times with KRPG buffer
- Then packed erythrocytes were suspended in 4 volumes of KRPG.
- Suspended erythrocytes were incubated with different concentrations of Withania Somnifera extract (1-10,000 µg/ml of DMSO).
- Erythrocytes were washed 2-3 times with KRP buffer to get packed RBC
- PRBC was used for MDA estimation

MDA Estimation-

0.2 ml packed erythrocytes were suspended in 3 ml of KRP buffer.
Then 1 ml of lysate was added to 1 ml of 10 % TCA (for ppt. of protein) and centrifuged for 5 minutes at 1000g.
1 ml of supernatant was added to 1 ml of 0.6 % TBA in 0.05 M/l of NaOH and boiled for 20 minutes at a temperature greater than 90ºC.
Then the solution was cooled and absorbance was taken at 532 nm and 630 nm as OD1 and OD 2.
Calculation-
O.D 2 – O.D 1 = Final O.D
A parallel experiment was performed using MDA standards at a concentration of (10^{-12} - 10^{-2})
Results were calculated from the reading of known MDA standards.

III. Result and Discussion

1. Effect of Withania Somnifera extract on Hydrogen peroxide (H_2O_2)

W.S. extract (1-10,000 µg/ ml) showed a significant increase in the percent decomposition of H2O2.
W.S. extract showed a low percent decomposition of H2O2 at lower concentrations and a high percent decomposition of H2O2 at higher concentrations.
Our results showed that erythrocytes' percent decomposition of H2O2 increases with increasing concentration of W.S extract.
Table No. 1

Table No. 1 Conc. Of W.S. (µg/ml)	% decomposition of H_2O_2
1	50 ± 2.0
10	66.6 ± 2.6
100	74 ± 3.7
1000	80 ± 2.4
10,000	83.3 ± 3.3

Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.

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Figure No. 1

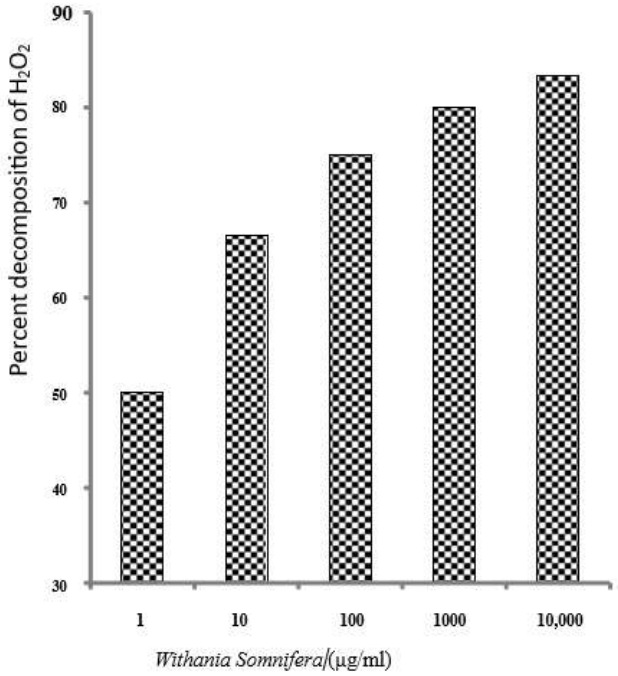

Figure No. 1: - Effect of Withania Somnifera extract on decomposition of H2O2 results are expressed as percent decomposition.

2. Effect of Withania Somnifera extract on Hydroxylation (OH)

W.S extract (1-10,000 µg/ ml) showed a significant increase in percent inhibition of hydroxylation. Our results showed that erythrocytes' percent inhibition of hydroxylation increases with increasing concentration of W.S extract.

Table No. 2

Conc. Of W.S. (µg/ml)	n mole of hydroxylated product	% inhibition of hydroxylation
1	61 ± 3.8	71.681
10	55 ± 2.2	78.112
100	51.5 ± 2.9	80.740
1000	53.3 ± 3.0	91.771
10,000	71 ± 4.2	95.143

Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.

Figure No. 2

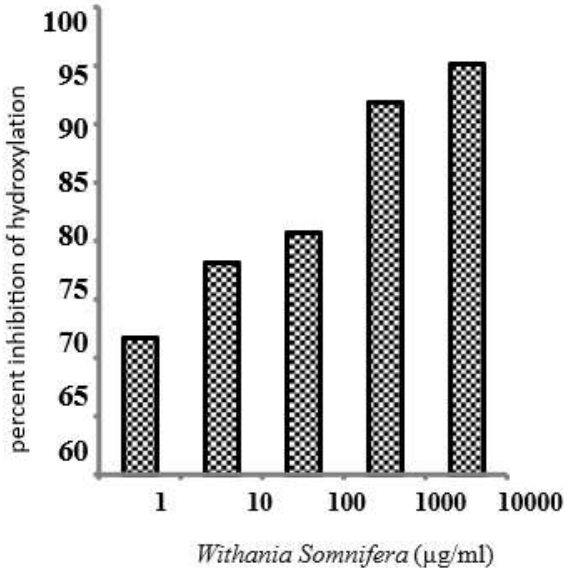

Figure No. 2: - Effect of Withania Somnifera extract on percent inhibition of hydroxylation results are expressed as percent inhibition.

3. Effect of Withania Somnifera extract on Osmotic fragility of erythrocyte: -

Our studies show that Withania Somnifera extract at a concentration of (10- 1000 µg/ml) exerts a significant protective effect on the OF or human erythrocytes. Figure No. 3.

The mean erythrocyte fragility (MEF i.e. concentration of PBS which caused 50 percent hemolysis) of erythrocytes treated with different concentrations of Withania Somnifera extract is shown in table no.3.

Maximum protection of osmotic fragility (decreased MEF) is observed at (100µg/ml) Withania Somnifera whereas no significant protection was observed at 1µg/ml. Withania Somnifera at a higher concentration of 10000µg/ml caused an increase in OF as compared to normal control.

Table No.3: -

Conc. Of W.S. (µg/ml)	Percent NaCl. Mean erythrocyte fragility (MEF)
Normal	0.690 ± 0.027
1	0.685 ± 0.034
10	0.680 ± 0.020
100	0.675 ± 0.033
1000	0.680 ± 0.027
10,000	0.795 ± 0.023

Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.

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Figure No. 3

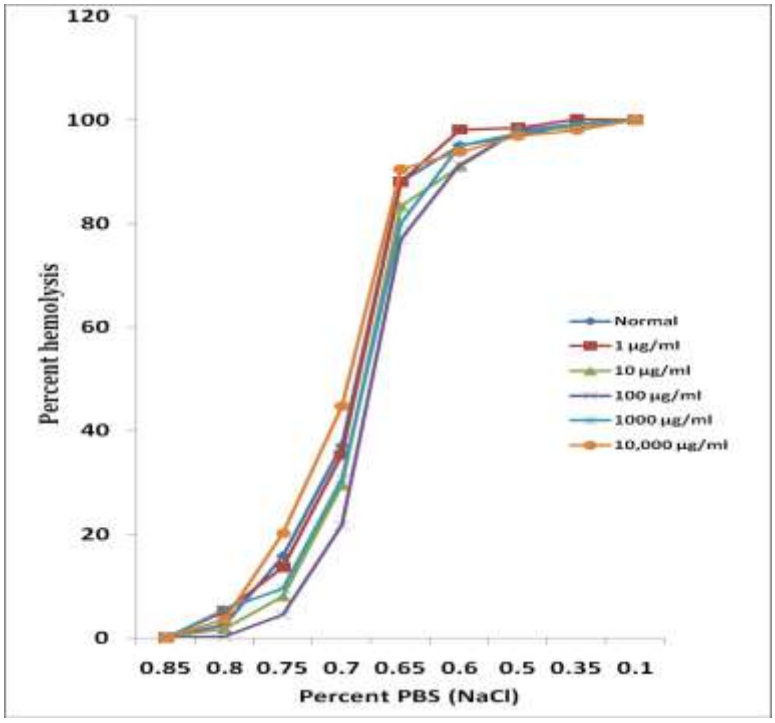


Figure No. 3: - Effect of Withania Somnifera extract on erythrocytes osmotic fragility results are expressed in percent hemolysis.

4. Effect of Withania Somnifera extract on Malondialdehyde (MDA)

W.S extract (10-1000 µg/ ml) showed a significant decrease in MDA level of RBC.

W.S. at a higher concentration of 10,000 µg/ ml showed a significant increase in the MDA level as compared to normal control.

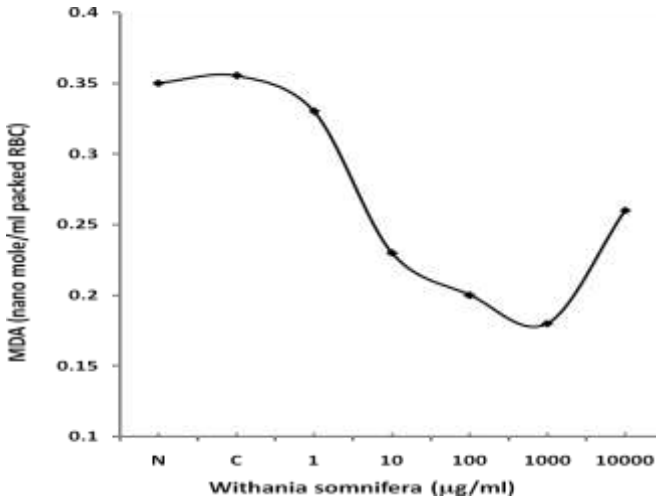
Our results showed that erythrocyte MDA level decreases with increasing concentration of W.S. up to Conc. of 1000 µg/ ml but further increase in W.S. Conc. increased in MDA level.

No significant effect of W.S. was observed at lower conc. of 1 µg/ ml on the MDA level.

Table No. 4: -

Conc. of W.S. (µl/ ml)	Conc. of MDA/ ml of PRBC
Normal	0.35 ± 0.021
Control	0.36 ± 0.016
1	0.33 ± 0.021
10	0.23 ± 0.011
100	0.20 ± 0.017
1000	0.18 ± 0.008
10,000	0.26 ± 0.013

Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.
Figure No. 4
Figure No. 4: - Effect of Withania Somnifera extract on MDA level of erythrocyte results are



expressed in n mole/ml of packed RBC.

VIII. CONCLUSION

The present study was undertaken to evaluate the antioxidant effect of Withania Somnifera. Antioxidant and free radicals scavenging activities in Extract of Withania Somnifera on RBC was studied in-vitro.
Based on our study shows:
Withania Somnifera extract shows a dose-dependent increase of H_2O_2 decomposition and inhibition of hydroxylation (OH formation). This indicates that Withania Somnifera extract possesses effective antioxidant and hydroxyl radical scavenging properties.
Withania Somnifera at a concentration of 10-1000 µg/ml protects RBCs and their membrane from damage as evidenced by a decrease in Osmotic Fragility.
A decrease in lipid peroxidation (MDA content) and an increase in antioxidant status (GSH level) of erythrocyte was observed at Withania Somnifera extract concentration of 10-1000 µg/ml as compared to normal control.
Our result also showed that a much higher concentration of Withania Somnifera extract (10,000 µg/ml) showed adverse effects on erythrocytes OF and MDA.
From our study, we can conclude that the Withania Somnifera extract can act as a potential antioxidant and protect the erythrocytes from oxidative damage by either directly scavenging the free radical or activating other processes. This property of Withania Somnifera may be due to its antioxidant features because Withania Somnifera contains many antioxidant compounds including (Somniferine, Somnine, Withanaine, Somniferinine, Withanolides, etc.) which are known antioxidants. But its indiscriminate use especially at higher concentrations should be avoided. As Withania Somnifera at higher concentration itself can cause generation of free radicals. Further studies are needed to elucidate its mechanism of action and its cytotoxic properties.

IV. REFERENCES:

[1] Abraham Z, Singh AK: In Dictionary of Indian Medicinal Plants Central Institute of Medicinal and aromatics plants, Lucknow, India 1992.
[2] Aust SD, Morehouse LA, and Thomas CD: Role of metals in oxygen radical's reactions, J. Free Rad. Biol Med 1985: 1:3-25.

[3] Bors. W, Suran M and Tait D: Oxygen Radicals in Chemistry and biology, W. De Gruyter, Berlin, New York. 1984.
[4] Basha PS and Rani AU. Cadmium-induced antioxidant defense mechanism in fresh water teleost Oreochromis massambicus (Tilapia). Ecotoxicol Environ saf. 2003 Oct, 56(2): 218-21.
[5] Beutler E. A manual of Biochemical method.Grunne and Stration, New York. 1984.
[6] Crichton RR: Interaction between iron metabolism and oxygen activation. In Oxygen Free Radical and Tissue Damage. Ciba Foundation Symposium 65. Experta Medica 1979, 57-76.
[7] Chan TK, Lacelle PL and Weed RL. Cell Physiol. 1975, 85:47.
[8] Concetti A, Massei P, Rotilio G, Brunori M, Rachmilewitz EA; Superoxide dismutase in red blood cells: method of assay and enzyme content in normal subjects and in patients with beta-thalassemia (major and intermedia); J Lab Clin Med. 1976 Jun;87(6):1057-64.
[9] Del Maestro R.F.: An approach to free radicals in medicine and biology: Aeta Physiol Scand 1980; 492: 155-168.
[10] Esterbauer H and Cheeseman KH. Determination of aldehydic lipid peroxidation product: Malondialdehyde and 4-hydroxynonenal method Enzymol. 1090; 186: 407-13.
[11] Freeman BA, Crapo JD: Biology of disease: Free radical and tissue injury. Lab Invest 1982; 47:412-426.
[12] Fridovich I. Oxygen radical, hydrogen peroxide and oxygen toxicity. In: free redical in biology (Pryor) 1: acadmic prees. NYP.1976. 239-277.
[13] Fridovich I. Superoxide anion radical superoxide dismutase and related matter. J.Biochem. 1997, 272-18525-18527.
[14] Fire B, Stocker R, Ames BN: Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natal Accad Sci. USA 1988; 85: 9748-9752.
[15] Fridovich I: Superoxide dismutases. Annu Rev Biochem. 1975; 44: 147-159.
[16] Gutteridge, Halliwell and Westernmarck: Free radical and biology: oxygen radical damage is biological system 1985, 121.
[17] Gergory EM, Fridovich I: Oxygen toxicity and Superoxide dismutase. J. Bacteriol 1973b; 114: 1193-1197.
[18] Ghosal S.; "Natural Products with Immunological Effects", Indian J. Indigenous Medicine 8, 1-7 (1991).
[19] Gay Men‟s Health crisis: Treatment issues, volume 7 no. 11/12-winter, 1993/94.
[20] Halliwell B, Gutteridge JMC and Cross CE: Free radicals, Antioxidants, and human disease: Where are we now? J Lab cin med 1992: 119(6): 598-620.
[21] Hallliwell B and Gutterige JMC. Method Enzymol. 1990, 186, 1-80.
[22] Halliwell B and Gutterige JMC. Iron and Free radical reactions: two spects of antioxidant protection. Trends Biochem science. 1989; 11: 372-375.
[23] J Vidyasagar, N Karunakar, MS Reddy, K Rajnarayana, T Surender, DR Krishna: Oxidative stress and antioxidant status in acute organophosphorus insecticide poisoning; year: 2000, 36; 2: 76-79.
[24] Jong kind JF, Verkerk A and Baggen RG. Free Radic. Biol. Med. 1989, 7(5): 507.
[25] Kuppurajan K, et.al. Effect of ashwagandha (Withania Somnifera Dunal) on the process of aging in human volunteers. J Res Ayurveda Siddha. 1980. 1: 247–258.
[26] Kondo T, Dale GL and Beulter E. Biochim. Biophys, Acta. 1981, 645:132.
[27] Kahl R. Synthetic antioxidant: Biochemical action and interference with radition, toxin compounds, chemical mutagens, and chemical carcinogens. Toxicology. 1984 Dec; 33(3-4): 185-228.
[28] Loto V, Pati A, Phatak A, Chandra N. Free radical, antioxidant and functional food: Impact on Human health. 2010, 4; 8: 118-126.
[29] Lakshmi-Chandra Mishra, Betsy B. Singh, Simon Dagenais; Scientific basis for the Therapeutic Use of Withania Somnifera (Ashwagandha). Altern Med Rev 2000; 5(4) 334-346.

[30] Mentzer WC and Clark MR. Biomembrane, vol 2, Pathological Membrane, ed. A. Novonty, Plenum press, New York. 1983, 79.
[31] Mamede AC, Tavares SD, Abrantes AM, Trindade JC, Maia Pereira JM, Botelho MF. The Role of Vitamins in Cancer: A Review. Nutr. Cancer. 2011 May 2:1-16.
[32] Maclennan DH, Branolt CJ, Korezal B and Green RG. Nature. 1985, 316: 696.
[33] Prakash D, Suri S, Upadhyay G and Singh BN. Total phenol, antioxidant and free radical scavenging activities of medicinal plant. Int J Food Scin Nutr. 2007 Feb; 58(1): 18-28.
[34] Rajangam Udayakumar & Sampath Kasthurirengan & Ayyappan Vasudevan; Antioxidant Effect of Dietary Supplement Withania Somnifera L. Reduce Blood Glucose Levels in Alloxan-Induced Diabetic Rats; Plant Foods Hum Nutr. 2010, 65:91–98, DOI 10.1007/s11130-009-0146-8.
[35] Rifikind JM, Araki K and Hardley EC.Arch, Biochem, Biophys. 1983, 222: 582.
[36] Sum Y: Free radicals; antioxidant: Free radical Biology and medicine 1990, vol. 8, 583.
[37] Smith Barbara: Natural Antioxidants. 2010.
[38] S. Manoharan, K. Kolanjiappan, K. Suresh & K. Panjamurthy. Lipid peroxidation & antioxidants status in patients with oral squamous cell carcinoma. Indian J Med Res 122, December 2005, pp 529-534.
[39] Sharma Shasikant. Biochemical studies on Antioxidant property of tithonia diversifolia, (Biochemistry department) 2003-2004.
[40] Srivastava SK and Beulter E. Biochem, Biophys. Res. Commun. 1969, 28: 659.
[41] Tang H, Qin Y, Li J and Gang X. The scavenging of superoxide radicals' apoptosis by novel cell-permeable fusion protein, STRAIL: FeSOD, in tumor necrosis factor related apoptosis-including-resistant leukemia cells. BCM Biol 2011 Mar 19; 9: 18.
[42] Tan YS, Webster RD. Electron-Transfer Reactions between the diamagnetic Cation of α-Tocopherol (Vitamin E) and β-Carotene. J Phys Chem B. 2011 Apr 14; 115(14): 4244-50.
[43] Tokio Matsunami, Yukita Sato et.al. Enhancement of reactive oxygen species and induction of apoptosis in streptozotocin –induced diabetic rates under hyperbaric oxygen exposure. Int J. Clin Exp Pathol. 2011 March 31; 4(3): 255-266.
[44] Uemi M, Ronsein GE, Prado FM, Motta FD, Miyamoto S, Medeiros MH, Di Mascio P. Cholesterol Hydroperoxides Generate Singlet Molecular Oxygen [O(2) ((1)Δ(g))]: Near-IR Emission, (18)O-Labeled Hydroperoxides, and Mass Spectrometry. Chem Res Toxicol. 2011 May 5.
[45] Vincent E. Giuliano. Protection against radiation. The second line of defense. 2008, Nov 17.
[46] Wood JL. In metabolic conjugation and metabolic hydrolysis (Fishman WD ed.) Academic press, New York. 1970: 2; 261-299.
[47] Wills, E.D. Mechanics of lipid peroxide formation in animal tissue; Biochem J 1966; 99:667.
[48] Wenger FA, Kilian M, Ridders J, Stahlknecht P, Schimke I, Guski H, Jacobi CA, Müller JM. Influence of antioxidative vitamins A, C and E. On lipid peroxidation in BOP-induced pancreatic cancer in Syrian hamsters. Prostaglandins Leukot Essent Fatty Acids. 2001 Sep; 65(3): 165-71.
[49] William A. Pryor. Free radicals in Biology 1994, 1; 2-11.
[50] Widodo N, Priyandoko D, Shah N, Wadhwa R and Koul SC. Selective Killing of cancer cells by Ashwagandha leaf extract and its component Withanone involve ROS signaling. PLoS One. 2010 Oct 21; 5(10): e 13536.
[51] Zhonqquo Zhang Xi Yi Jie He Za Zhi. Effect of promoting blood circulation to remove statsis and supplementing qi and yin on the lipid peroxide and superoxide dismutase during experimental pulmonary fibrosis. 1994 Dec; 14(12): 733-5.