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EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF SOME NUTRITIONAL SUPPLEMENTS

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Keywords:

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ABSTRACT: The nutritional supplements are the natural food supplements, and other related products used to boost the nutritional content of the diet. These nutritional supplements have been of interest to research because of their antioxidant property by consumption of food and drinks. The present study deals with a comparative evaluation of antioxidant and anti-inflammatory effects of marketed nutritional supplements using cytoprotection, lipidperoxdiation and membrane stabilization activities on RBC under in-vitro condition. The marketed nutritional supplements exhibited cytoprotection or prevented hemolysis in erythrocytes and provided better protection against H_2O_2 induced oxidation and lipid peroxidation and exhibited good membrane stabilization when compared to standard. The Women Horlicks showed significant protection against H_2O_2 induced cytotoxicity in erythrocytes when compared to other supplements, the nutritional supplement Boost provided better protection against lipidperoxidation induced by H_2O_2 in erythrocytes. The nutritional supplement Maltova showed better membrane stabilization activity when compared to standard and other nutritional supplement.

INTRODUCTION: Nutrition can be defined as all processes used by an adult or child to take in food and to digest, absorb, transport, utilize, and excrete food substances, components or substances found in food are called as nutrition [1]. Nutritional supplements include vitamins, minerals, herbs, meal supplements, sports nutrition products, natural food supplements, and other related products used to boost the nutritional improve the content of the diet. Nutritional supplements are used for many purposes.

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They can be added to the diet to boost overall health and energy; to provide immune system support and reduce the risks of illness and age-related conditions; to improve performance in athletic and mental activities; and to support the healing process during illness and disease [2]. However, most of these products are treated as food and not regulated as drugs. Nutritional supplement drinks are available for different purposes and different age groups children's, women's, sport persons, diseased persons, pregnant ladies, feeding mothers [2].

In present study antioxidant, cytoprotection and membrane stabilization activities of different nutritional supplements was studied on RBC.

MATERIALS AND METHODS: Human red blood cell, Saline phosphate buffer containing 0.44

mol/l H_2O_2**,** TCA containing 0.1 M sodium Meta aresenite, thiobarbituric acid (TBA), malondialdehyde (MDA), 1,1,3,3-tetra ethoxypropane (**TEP),** ascorbic acid, hydrogen peroxide, hypotonic saline, isotonic saline, diclofenac sodium, sodium hydroxides.

Nutritional supplements:
Boost, Horlicks, Women Horlicks, Junior Horlicks and Kissan Nutria Gro (Glaxo Smith Kine, Punjab), Complan Memory, Complan Nutri Gro, Complan Kesar Badam (Heinz India Pvt Limited, Mumbai) Pediasure (Abbott Singapore Private Limited, Singapore), Bournvita, Bournvita 5 Star (Cabdury India Limited, India), Protinex Vanilla (Wock Hardt Limited, Mumbai).

Methodology:
Sample preparation:
700mg of nutritional supplements was transferred into a beaker and 5ml of distilled water was added and mixed well [4].

Lipid peroxidation method:
An aliquot containing 1ml of RBC suspension in 9 ml saline phosphate buffer(pH 7.2) which contains 0.44 mol/L H_20_2 was prepared. To it 1ml of test sample was added. About 2.5ml aliquot was withdrawn at zero hour and remaining aliquot (2.5ml) was incubated at 37**°** for 2 hours. To all the tubes 1 ml of 28% trichloroacetic acid (TCA) was added and kept aside. The 3 ml of supernatant was taken and to it 1ml of 1% thiobarbituric acid (TBA) was added and boiled for 15mins. The tubes were then cooled and centrifuged for 15 min at 3000 rpm. Absorbance was read at 540 nm using Shimadzu spectrophotometer (UV-1800). All the determinations were performed in triplicate. The malondialdehyde (MDA) content was estimated [3, 4].

Cytoprotection activity:
The samples were prepared as shown in **Table 1**.

TABLE 1: CYTOPROTECTION ACTIVITY

Test	Positive control	Negative control	Standard
2ml nutritional supplements(140mg/ml)+ 400µl RBC suspension	2ml Ascorbic acid (140mg/ml)+ 400µl RBC suspension	2ml phosphate buffer solution (p^H 7.2)+400µl RBC suspension	5.6ml H_2O_2 (10mM)+400µl RBC suspension
1.6ml PBS (pH 7.2)+2ML H_2O_2 (10mM)	1.6ml PBS (pH 7.2)+2ML H_2O_2 (10mM)	1.6ml PBS (pH 7.2)+2ML H_2O_2 (10mM)	-

To the test, positive control, negative control, RBC suspension was added and incubated for 1 hr. Phosphate buffer and hydrogen peroxide were then added to the above solution and incubated for 1½ hr and the supernatant solutions were separated.

The hemoglobin released into the supernatant of the mixtures was measured at 540 nm using Shimadzu spectrophotometer (UV-1800). All the determinations were performed in triplicate [5].

$$\text{Percentage Cytoprotection activity} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of H2O2}} \times 100$$

Membrane stabilization:
Test solution: A reaction mixture consisting of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4), 0.5ml of test sample, 0.5ml of 10%w/v human red blood cell in isotonic saline was prepared and finally the volume was made up to 4.5ml with hypotonic saline**.**

Blood control:
A control mixture consisting of 2.5ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4), and 0.5ml of 10%w/v human red blood cell in isotonic saline was prepared and finally the volume was made up to 4.5ml with hypotonic saline**.**

Standard solution:
A standard mixture containing 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4), 0.5ml of diclofenac sodium, and 0.5ml of 10%w/v human red blood cell in isotonic saline was prepared and finally the volume was made up to 4.5ml with hypotonic saline.

Drug control:

A reaction mixture containing 2.5ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4), and 0.5ml of diclofenac sodium solution was prepared and finally the volume was made up to 4.5ml with hypotonic saline [7].

The mixtures were incubated at 56°C for 30 minutes. The tubes were cooled under running water for 20 minutes, and mixtures were centrifuged at 3000 rpm for 10min and the supernatant liquid was separated. The absorbance of supernatant was read at 560 nm using shimadzu spectrophotometer (UV-1800). All the determinations were performed in triplicate.[6, 7]

$$\% \text{ stabilizing activity} = \frac{\text{Abs of test-abs of control}}{\text{Abs of blood control}} \times 100$$

RESULTS AND DISCUSSION:

Effect of different extracts on lipid peroxdiation:

Extent of lipid peroxidation is measured through malondialdehyde levels in the biological tissues and fluids, a pro-oxidant factor which determines the oxidative damage.

The standard graph of malondialdehye for determination of lipid peroxide levels is shown in **Fig 1**. The absorbance values and concentrations were found to be linear in the concentration range of 1-30 nM/ml, as confirmed by the correlation coefficient of 0.994. The absorbance (y) was proportional to the concentration of Ascorbic acid (x) following the regression equation y=0.0298+0.0378.

The results reveal that most of the nutritional supplements provided better protection against lipid peroxidation induced by H_2O_2 in erythrocytes. The nutritional supplements Women Horlicks, Proteinex V, Proteinex C, Kissan smart exhibited better results in lipid peroxidation induced by H_2O_2 in erythrocytes. (**Table.2, Fig.1** and **Fig.2**)

TABLE 2: EVALUATION OF LIPID PEROXIDATION ACTIVITY OF NUTRITIONAL SUPPLEMENTS

S.no	Products	MDA levels at 0 –Hour(Nm)	MDA levels at 2 –Hour (Nm)
1	Protenix.C	2.89±0.1	10.87±0.97
2	Protenix.V	4.75±0.33	9.61±0.22
3	Horlick.W	4.10±1.01	7.68±0.84
4	Horlick.J	7.49±0.67	12.6±0.51
5	Horlick	13.61±0.96	17.86±2.37
6	Bournvita	11.15±0.67	14.335±1.18
7	Pedisure	11.59±0.196	13.60±0.773
8	Kissansmart	9.02±0.702	11.59±0.19
9	Bournvita 5 Star	15.29±0.51	17.6332±1.65
10	Complan.K.B	14.06±1.174	20.65±1.17
11	Complan.M	16.74±0.840	24.01±1.400
12	Complan. N.G	20.32±1.40	22.8±1.00
13	Maltova	17.19±1.34	19.87±1.537
14	Boost	16.41±0.509	20.78±1.405
15	Ascorbic Acid	3.85±0.517	2.35±0.08

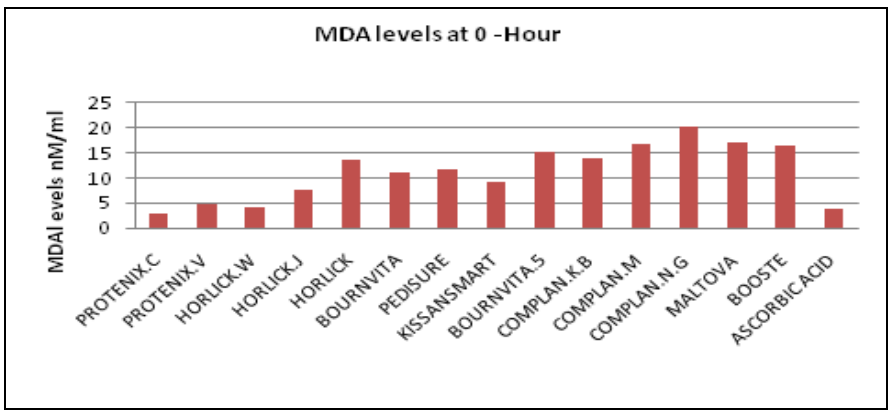

FIG.1. EVALUATION OF LIPIDPEROXIDATION ACTIVITY OF NUTRITIONAL SUPPLEMENTS AT 0-HOUR

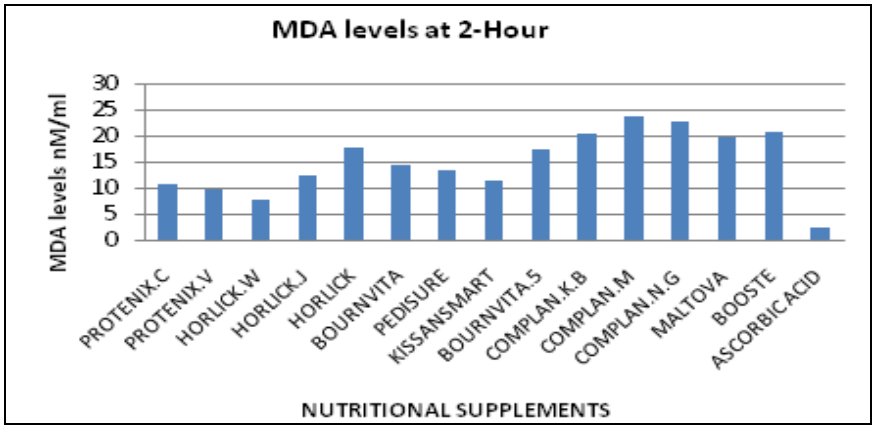

FIG. 2: EVALUATION OF LIPID PEROXIDATION ACTIVITY OF NUTRITIONAL SUPPLEMENTS AT 2-HOUR

Cytoprotection activity:

According to our results, the percentage of hemolysis in erythrocytes pretreated with the nutritional supplements was lower than the non pretreated erythrocytes suspension. Nutritional supplements like Women Horlicks and Pediasure showed significant protective effect against H_2O_2 induced cytotoxicity in erythrocytes. (**Fig.3** and **Table 3**)

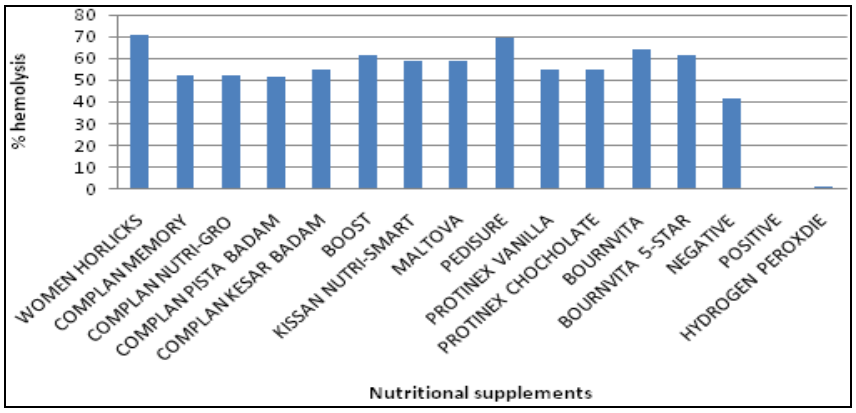

FIG. 3: EFFECT AND PERCENTAGE OF DIFFERENT NUTRITIONAL SUPPLEMENTS ON CYTOTOXCITY

TABLE 3: CYTOPROTECTION AGAINST HYDROGEN PEROXIDE INDUCED ON HUMAN RBC

Cytoprotection by hydrogen peroxdie induced human RBC		
S.No	**Product**	**% Hemolysis**
1	Horlick	61.50
2	Junior Horlicks	63.50
3	Women Horlicks	71.00
4	Complan Memory	52.00
5	Complan Nutri-Gro	52.00
6	Complan Pista Badam	51.56
7	Complan Kesar Badam	55.00
8	Boost	61.50
9	Kissan Nutri-Smart	59.10
10	Maltova	59.10
11	Pedisure	69.20
12	Protinex Vanilla	54.80
13	Protinex Chocholate	54.80
14	Bournvita	63.90
15	Bournvita 5-Star	61.50
16	Negative	41.45
17	Positive	92.80
18	Hydrogen Peroxdie	100

Membrane stabilization method:

The percentage membrane stabilization of different nutritional supplements is shown in **Table 4**. The results indicate that the Maltova had highest *in-vitro* anti-inflammatory activity when compared to other products. (**Fig.4** and **Table.4**)

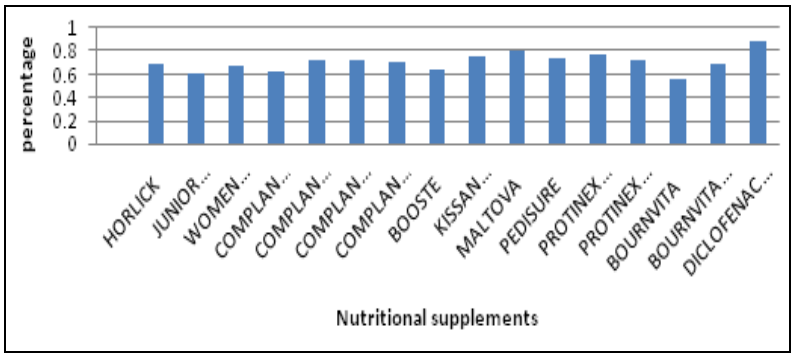

FIG.4: PERCENTAGE OF MEMBRANE STABILIZATION OF DIFFERENT PRODUCT

TABLE 4: EFFECT OF DIFFERENT NUTRITIONAL SUPPLEMENTS ON MEMBRANE STABILIZATION

Membrane stabilisation method				
S.no	**Product**	**Average reading**		**Percentage (%)**
		Test control	**Drug control**	
1	Horlick	1.986	0.225	68.46
2	Junior Horlicks	2.125	0.441	61.07
3	Women Horlicks	2.109	0.352	67.58
4	Complan Memory	2.621	0.926	62.05
5	Complan Nutri-Gro	2.801	0.998	71.70
6	Complan Pista Badam	2.805	0.997	71.85
7	Complan Kesar Badam	2.457	0.668	70.45
8	Booste	2.254	0.542	63.57
9	Kissan Nutri-Smart	2.409	0.561	75.70
10	Maltova	2.75	0.85	80.36
11	Pedisure	2.926	1.523	73.00
12	Protinex Vanilla	2.295	0.426	77.58
13	Protinex Chocholate	2.265	0.448	72.94
14	Bournvita	2.097	0.469	56.07
15	Bournvita 5-Star	1.996	0.22	69.29
16	Diclofenace Sodium	2.108	0.120	88.2

Summary: The results of the present investigation reveal that nutritional supplements like Horlicks and Bournvita exhibited the better antioxidant potential. Nutritional supplements like Protienx, Horlicks Junior, and Complan exhibited good protection against lipid peroxdiation induced by H_2O_2. Women Horlicks and Pediasure showed significant protection against H_2O_2 induced cytotoxicity in erythrocytes. Maltova had highest *in-vitro* anti-inflammatory activity when compared to other products.

CONCLUSION: The present study reveals that nutritional supplements were found to active against cytotoxicity, oxidation, and membrane destabilization**.** The comparative data obtained regarding the pharmacological activities of nutritional supplements in the present investigation can be used for further studies in other areas of interest and also for studies on healthy volunteers.

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