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"Evaluation of Effect of Amalaki Churna (Emblica Officinalis Gaertn.) As Rasayana and Its Free Radicals Scavenging Activity in Healthy Individuals on Basis of Time of Administration of Medicine" by Superoxide Dismutase Test (SOD)

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Abstract--- *The present study was carried out in Parul Institute of Ayurved, Parul University, Vadodara, Gujarat, India. Amalaki fruits were collected from Khanderao Market, Vadodara, Gujarat, India. The Amalaki fruit was authenticated at The Maharaja Sayajirao University of Baroda, Department of Botany, Faculty of Science, Vadodara- 390 002, Gujarat, India and sample code number was compared with (BARO 123450018408, 18415). Main objective of the study is to screen comparative study of Amalakichurna (Emblica officinalis Gaertn.) as Rasayana in three different Oushadhasevan kala i.e. time of administration of medicine as Kinchitsuryodayajate (Sun-rise), Divas bhojane (Midday meal), Nishi (Night meal), and Regular food is given to Control group.*

Study design: *A total of 100 healthy individual were selected and divided into 4 groups.*

Control group: *Regular food was given for 30 days*

Group 1; *Amalaki churna was given for 30 days during (Kinchit Suryodayajate)during sunrise process time (06:00 a.m.)*

Group 2: *Amalaki churna was given for 30 days at Midday meal time (12:00 pm)*

Group 3: *Amalaki churna was given for 30 days at Night time (08:00 p.m.)*

Superoxide dismutase was assayed in all the study groups by the method devised by Marklund S, Marklund G modified by Nandi and Chatterjee. Blood samples were collected from all the subjects. Analysis of study was done by using Tukeys multiple posthoc procedure and one way ANOVA test. Result- The serum SOD levels were significantly decreased in group 1 (8.09 units/ml.) as compared to control (14.37 units/ml.), group 2 (9.74 units/ml.) and group 3 (9.77 units/ml.) respectively. Conclusion- These results provide enough evidence of increased oxidative stress and a compromised antioxidant defense system in groups of healthy individuals.

Keywords--- *Amalakichurna, SOD, Free Radicals Scavengers, Healthy Individuals, Rasayana, Time of Administration.*

I. INTRODUCTION

Oushadhasevan kala (time of administration of medicine) is one of the important factor in treatment aspect.[1]

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Rasayana Tantra branch deals with delay in the process of aging, increases life span, medha i.e. intellect, bala i.e. strength and also increases the natural immunity of the body.[2] The Rasayan as are used to promote health and longitivity by increasing defense against diseases, arresting the aging process and revitalizing the body in debilitated conditions[3]. The clinical efficacy of the fruits of *Emblica officinalis* Gaertn. Are described in Ayurveda and Amalaki is referred to as a best Vayasthapana (causing rejuvenation) drug.

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy should, therefore, include either natural free radical scavenging antioxidant enzymes against which capable of augmenting the activity of these enzymes which include SOD (Superoxide Dismutase), CAT (Catalase), LPO (Lipid Peroxide)[4]. If human disease is considered to result from an imbalance between oxidative stress & antioxidant defense, then it is conceivable that it may be possible to limit oxidative tissue damage & hence prevent or ameliorate disease progression by supplementing antioxidant defense. By virtue of their properties & clinical use in Ayurveda, the Rasayana may provide potential therapeutic intervention against oxidative threats both in healthy and disease condition.[5]

Table 1: Schedule of the proposed research work: 4 Groups of 25 healthy individuals are taken in each group.

Group	**Drug**	**Time**	**Dosage form**	**Days**
Group 1	Amalaki churna	Kinchit Kinchit Suryodayajate(Sun-rise time 06**:**00 A.M.)	Churna	30
Group 2	Amalaki churna	Divas bhojane(Midday meal 12**:**00 P.M)	Churna	30
Group 3	Amalaki churna	Nishi(Night meal 08**:**00 P.M)	Churna	30
Group 4	Food	Regular	---------	30

Amalakichurna (*Emblica officinalis* Gaertn.) was given to all the groups of healthy individuals except control group. In first group Amalaki churna was given at Kinchit Suryodayajate (during sunrise process), in second group Amalaki churna was given at Divas bhojane i.e. (Midday meal time), and in third group Amalaki churna was administered at Nishi (Night meal time) lastly control group was given with regular food. Strict time schedule is maintained throughout the procedure. Blood samples (nearly about 10 ml of each healthy individual) of all 100participants were taken in unbreakable non- vacuum blood collection tubes before starting the study and collected blood samples were transferred to centrifuge machine for centrifugation. After 5 minutes of centrifugation serum was collected at top (approximately about 5 ml) of the tubes, then serum was preserved in deep freezer at -80^0 C. Then from next day administration of Amalakichurna (*Emblica officinalis* Gaertn.) was started for 30 days. After completion of 30 days blood was collected in unbreakable non-vaccume tubes transferred it to centrifuge machine, collect the serum. Next to that readings were taken in microplate reader machine.

Fig. 1: Preparation of Amalakichurna(*Emblica officinalis* Gaertn.) with Sukhoshna Jala (Luke Warm Water).

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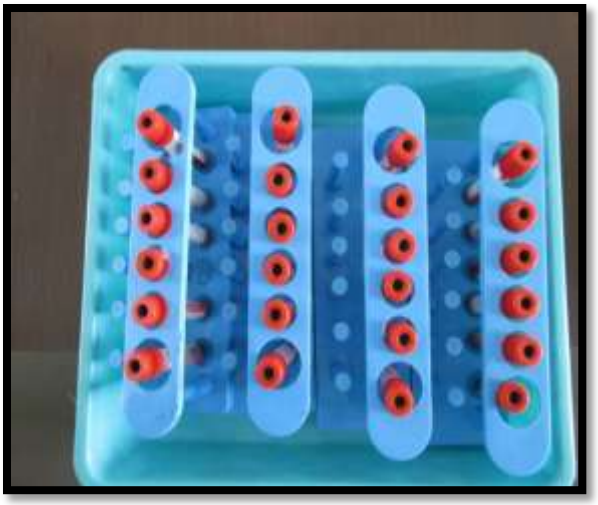

Fig. 2: Unbreakable non vaccume blood collection tubes

Fig. 3: Blood collections from Healthy Individuals

Fig. 4: Separation of serum from blood by centrifuge method

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Fig. 5: Preparation of serum samples before and after study for reading

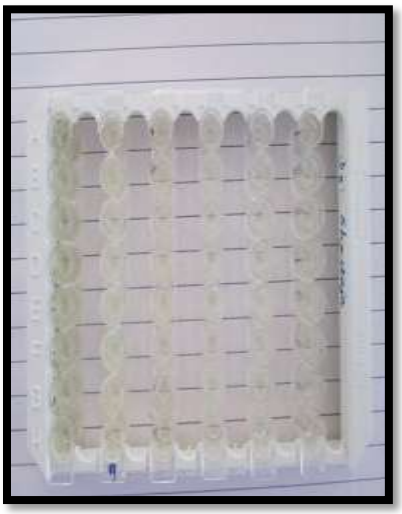

Fig. 6: SOD Kit

Fig. 7: Deep Freezer

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Fig. 8: Micro-plate reader machine

II. MATERIALS AND METHODS

Estimation of Serum Superoxide Dismutase

Superoxide dismutase was assayed in all the study groups by the method devised by Marklund S, Marklund G modified by Nandi and Chatterjee. Retro-orbital blood samples were collected from all the subjects.

Principle: [6]

Pyrogallolautooxidises rapidly in aqueous or alkaline medium solution and this has been employed for the estimation of superoxide dismutase. SOD inhibits the auto oxidation of pyrogallol. This principle was employed in a rapid and convenient method for the determination of the enzyme concentration.

Reagents

1. Tris Buffer

50 ml of Tris buffer (containing 50 mM of Tris buffer and 1 mM of EDTA) was prepared. To this, 50 ml HCL was added to adjust the pH at 8.5 and volume was made up to

100 ml.

2. Pyrogallol: (20 mM concentration)

25 mg of pyrogallol was dissolved in 10 ml of distilled water.

Procedure: [7]

For Control

To 2.9 ml of Tris buffer, 0.1 ml of pyrogallol solution was added, mixed and reading was taken at 420 nm, exactly after 1 minute 30 seconds and 3 minutes 30 seconds. The absorbance per two minutes was recorded and the concentration of pyrogallol was adjusted (by diluting the pyrogallol solution) so that the rate of change of absorbance per minute was approximately 0.020 – 0.023 nm.

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For Sample

To 2.8 ml of Tris buffer, 0.1 ml of serum sample was added, mixed and started the reaction by adding 0.1 ml of adjusted pyrogallol solution (as per control). It was read at 420 nm exactly after 1 minute 30 seconds and 3 minutes 30 seconds and absorbance per 2 minutes was recorded.

Calculations

Absorbance reading of control - A

Absorbance reading of sample - B

Units of SOD/3 ml of assay mixture = [(A-B) / (A×50)] ×100

Unit×10 = Units /ml of sample solution.

Definition of Unit

One unit of superoxide dismutase is described as the amount of enzyme required to cause 50 % inhibition of pyrogallol auto oxidation per 3 ml assay mixture.

Normal range

SOD in serum is 2.93-3.71 units/ml. Analysis of study was done by using Tukeys multiple posthoc procedure.

Table 2: Result: For Super Oxide Dismutase (S.O.D.) Test Before

Sample Code (Group 1)	Result Units/ml.	Sample Code (Group 1I)	Result Units/ml.	Sample Code (Group 1II)	Result Units/ml.	Sample Code (Group 1V)	Result Units/ml.
1.	11.1	26.	12.3	51	12.0	76	4.2
2.	4.3	27	9.6	52	13.2	77	11.3
3.	11.2	28	12.5	53	11.9	78	16.3
4.	15.2	29	11.6	54	11.0	79	7.9
5.	4.6	30	9.6	55	13.2	80	9.2
6.	7.4	31	11.4	56	8.0	81	13.0
7.	3.2	32	14.2	57	12.2	82	13.2
8.	9.6	33	16.1	58	9.5	83	19.0
9.	11.8	34	17.4	59	11.0	84	18.4
10.	5.3	35	11.9	60	8.9	85	17.6
11.	9.4	36	4.5	61	11.2	86	16.2
12.	11.3	37	8.0	62	9.5	87	9.1
13.	16.4	38	11.2	63	4.2	88	12.4
14.	9.7	39	9.5	64	12.5	89	13.3
15.	14.5	40	15.0	65	5.3	90	9.0
16.	15.8	41	17.2	66	9.4	91	11.7
17.	16.9	42	18.9	67	11.4	92	14.7
18.	11.9	43	17.5	68	16.4	93	16.5
19.	13.7	44	5.3	69	9.7	94	19.2
20.	8.9	45	9.4	70	14.5	95	8.8
21.	17.6	46	12.4	71	15.8	96	13.7
22.	9.5	47	13.4	72	4.9	97	14.6
23.	7.7	48	9.7	73	8.2	98	17.6
24.	17.4	49	12.5	74	11.3	99	9.5
25.	11.9	50	11.8	75	19.3	100	16.4

Super Oxide Dismutase Reference value: 2.93-3.71 units/ml

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Table 3: Result: For Super Oxide Dismutase (S.O.D.) Test After

Sample Code (Group 1)	**Result Units/ml.**	**Sample Code (Group 1I)**	**Result Units/ml.**	**Sample Code (Group 1II)**	**Result Units/ml.**	**Sample Code (Group 1V)**	**Result Units/ml.**
1.	10.1	26.	11.2	51	11.8	76	5.8
2.	3.2	27	8.2	52	9.5	77	10.3
3.	10.1	28	11.8	53	10.7	78	17.5
4.	13.1	29	10.6	54	10.3	79	9.9
5.	4.2	30	8.2	55	11.9	80	8.1
6.	3.9	31	10.7	56	7.1	81	14.3
7.	3.0	32	13.1	57	9.5	82	13.2
8.	8.3	33	15.9	58	8.7	83	19.8
9.	9.4	34	16.5	59	12.9	84	20.5.
10.	4.3	35	10.3	60	9.5	85	21.7
11.	8.2	36	3.7	61	11.2	86	17.2
12.	10.7	37	7.5	62	8.7	87	10.4
13.	11.3	38	10.2	63	3.3	88	12.3
14.	3.9	39	9.4	64	7.8	89	14.1
15.	2.9	40	8.5	65	3.4	90	10.9
16.	3.7	41	16.6	66	8.9	91	12.5
17.	3.1	42	15.8	67	10.2	92	15.3
18.	10.2	43	16.1	68	15.4	93	17.8
19.	11.2	44	3.7	69	8.6	94	20.2
20.	7.3	45	7.9	70	13.5	95	9.4
21.	16.3	46	8.5	71	14.3	96	14.8
22.	8.9	47	3.2	72	3.1	97	15.6
23.	6.2	48	7.7	73	7.7	98	19.9
24.	17.9	49	3.3	74	10.8	99	10.1
25.	10.8	50	4.8	75	15.4	100	17.7

Super Oxide Dismutase Reference value: 2.93-3.71 units/ml

III. RESULTS

Table 4: Comparison of four groups (I, II, III, IV) with pretest and posttest blood serum levels in super oxide dismutase (S.O.D.) chemical by one way ANOVA

Treatment time	Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Pretest	Between groups	3	90.03	30.01	2.0354	0.1141
	Within groups	96	1415.36	14.74		
	Total	99	1505.39			
Posttest	Between groups	3	548.2331	182.7444	11.0836	0.0001*
	Within groups	96	1582.8288	16.4878		
	Total	99	2131.0619			
Difference	Between groups	3	237.2684	79.0895	12.2437	0.0001*
	Within groups	96	620.1240	6.4596		
	Total	99	857.3924			

*$p<0.05$ indicates significant

From the results of the above table, it can be seen that,

- No significant difference was observed between four groups (I, II, III, IV) with respect to pretest blood serum levels in super oxide dismutase (S.O.D.) chemical ($F=2.0354$, $p=0.1141$) at 5% level of significance. It means that, the pretest blood serum levels in super oxide dismutase (S.O.D.) chemical are similar in four groups (I, II, III, IV).

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- A significant difference was observed between four groups (I, II, III, IV) with respect to posttest blood serum levels in super oxide dismutase (S.O.D.) chemical (F=11.0836, p=0.0001) at 5% level of significance. It means that, the posttest blood serum levels in super oxide dismutase (S.O.D.) chemical are different in four groups (I, II, III, IV).
- A significant difference was observed between four groups (I, II, III, IV) with respect to posttest blood serum levels in super oxide dismutase (S.O.D.) chemical (F=12.2437, p=0.0001) at 5% level of significance. It means that, the posttest blood serum levels in super oxide dismutase (S.O.D.) chemical are different in four groups (I, II, III, IV).

Further, if F is significant or not significant, to know the pair wise comparisons of four groups (I, II, III, IV) with respect to mean of pretest and posttest blood serum levels in super oxide dismutase (S.O.D.) chemical by applying the Tukeys multiple posthoc procedures and the results are presented in the following table.

Table 5: Pair wise comparison of four groups (I, II, III, IV) with pretest and posttest blood serum levels in super oxide dismutase (S.O.D.) chemical by Tukeys multiple posthoc procedures

Treatment time	Groups	Group I	Group II	Group III	Group IV
Pretest	Mean	11.05	12.12	10.98	13.31
	SD	4.22	3.62	3.50	3.97
	Group I	-			
	Group II	p=0.7614	-		
	Group III	p=0.9999	p=0.7230	-	
	Group IV	p=0.1668	p=0.6897	p=0.1459	-
Posttest	Mean	8.09	9.74	9.77	14.37
	SD	4.22	4.23	3.34	4.37
	Group I	-			
	Group II	p=0.4810	-		
	Group III	p=0.4640	p=1.0000	-	
	Group IV	p=0.0001*	p=0.0007*	p=0.0008*	-
Difference	Mean	2.96	2.38	1.21	-1.06
	SD	3.87	2.80	1.39	1.05
	Group I	-			
	Group II	p=0.8485	-		
	Group III	p=0.0770	p=0.3698	-	
	Group IV	p=0.0001*	p=0.0002*	p=0.0112*	-

*p<0.05

From the results of the above table, it can be observe the followings:

- No significant difference was observed between Group I and Group II, Group I and Group III, Group I and Group IV, Group II and Group III, Group II and Group IV; Group III and Group IV with mean pretest blood serum levels in super oxide dismutase (S.O.D.) chemical at 5% level of significance. It menas that, the mean pretest blood serum levels in super oxide dismutase (S.O.D.) are similar in all the pairs of groups.
- A significant difference was observed between Group I and Group IV, Group II and Group IV; Group III and Group IV with mean posttest blood serum levels in super oxide dismutase (S.O.D.) chemical at 5% level

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of significance. It menas that, the mean posttest blood serum levels in super oxide dismutase (S.O.D.) chemical is significantly higher in group IVas comapred to least in group I followed by other groups.

- But when comparing the pairs of groups with mean difference blood serum levels in super oxide dismutase (S.O.D.) chemical from pretest to posttest found to be significant between Group I and Group IV, Group II and Group IV; Group III and Group IV at 5% level of significance. It means that, the mean difference from pretest to posttest blood serum levels in super oxide dismutase (S.O.D.) chemical is significantly higher group I and least in Group IV followed by other groups. The mean of pretest and posttest blood serum levels in super oxide dismutase (S.O.D.) chemical is also presented in the following figure.

Graph No. 1 - Comparison of four groups (I, II, III, IV) with pretest and posttest blood serum levels in super oxide dismutase (S.O.D.) chemical

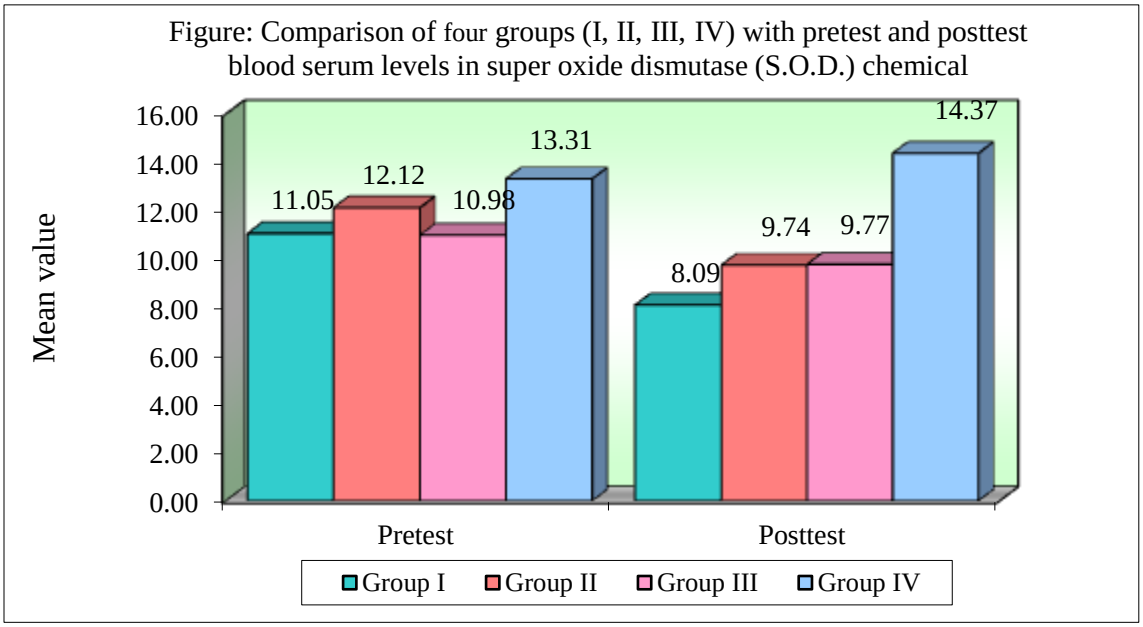

Table 6: Comparison of pretest and posttest blood serum levels in four groups (I, II, III, IV) of super oxide dismutase (S.O.D.) chemical by dependent t test

Groups	Time	Mean	Std.Dv.	Mean Diff.	SD Diff.	% of change	Paired t	P-value
Group I	Pretest	11.05	4.22					
	Posttest	8.09	4.22	2.96	3.87	26.82	3.8296	0.0008*
Group II	Pretest	12.12	3.62					
	Posttest	9.74	4.23	2.38	2.80	19.64	4.2529	0.0003*
Group III	Pretest	10.98	3.50					
	Posttest	9.77	3.34	1.21	1.39	11.04	4.3496	0.0002*
Group IV	Pretest	13.31	3.97					
	Posttest	14.37	4.37	-1.06	1.05	-7.96	-5.0707	0.0001*

*$p<0.05$

The results of the above table, it clearly shows that,

- A significant difference was observed between pretest and posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group I (t=3.8296, p=0.0008) at 5% level of significance. It means that, the posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group I are significantly higher

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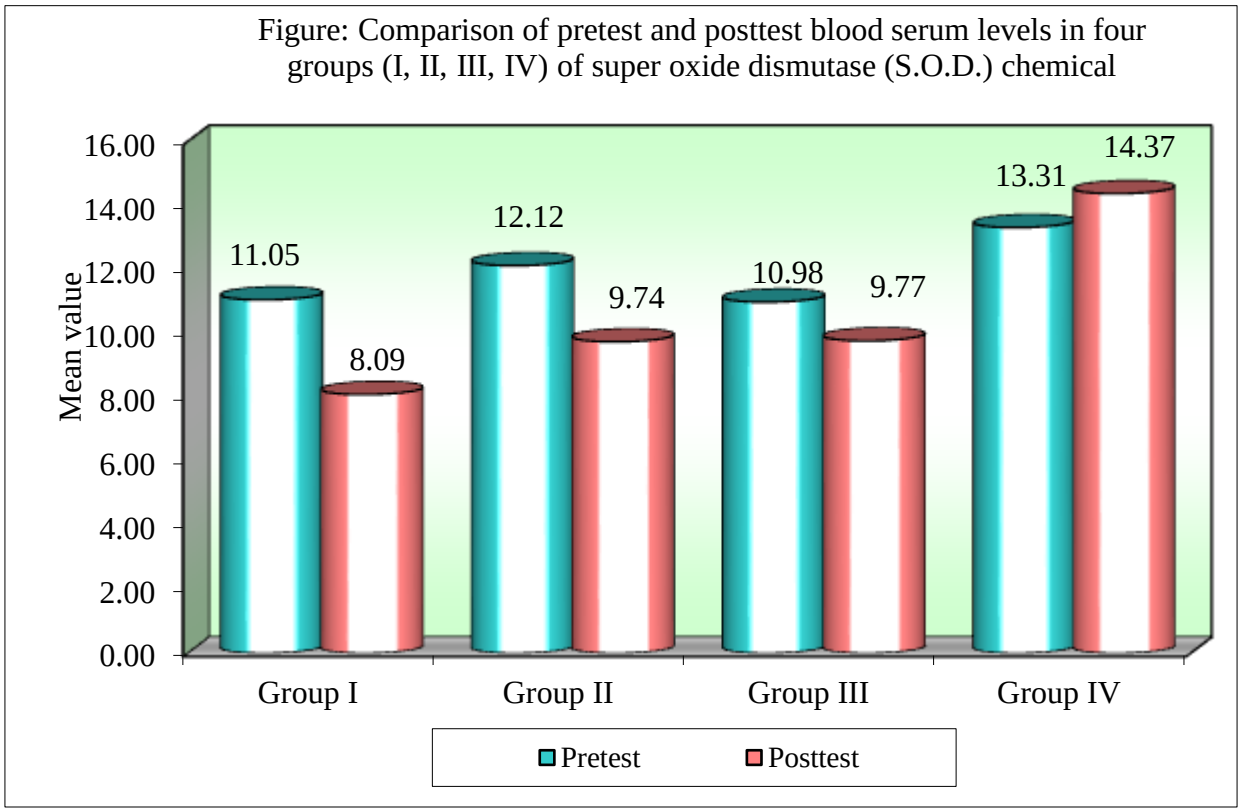
(about 26.82% decrease) as compared to pretest blood serum levels of super oxide dismutase (S.O.D.) chemical in group I.

- A significant difference was observed between pretest and posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group II (t=4.2529, p=0.0003) at 5% level of significance. It means that, the posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group II are significantly higher (about 19.64% decrease) as compared to pretest blood serum levels of super oxide dismutase (S.O.D.) chemical in group II.
- A significant difference was observed between pretest and posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group III (t=4.3496, p=0.9514) at 5% level of significance. It means that, the posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group III are significantly higher (about 11.04% decrease) as compared to pretest blood serum levels of super oxide dismutase (S.O.D.).
- A significant difference was observed between pretest and posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group IV (t=-5.0707, p=0.0002) at 5% level of significance. It means that, the posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in group IV are significantly higher (about 7.96% increase) as compared to pretest blood serum levels of super oxide dismutase (S.O.D.). The mean pretest and posttest blood serum levels of super oxide dismutase (S.O.D.) chemical in four groups are also presented in the following figure.

Graph No. 2 - Comparison of pretest and posttest blood serum levels in four groups (I, II, III, IV) of super oxide dismutase (S.O.D.) chemical



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IV. DISCUSSION

About 5% or more of the inhaled oxygen (O_2) is converted to reactive oxygen species (ROS) by univalent reduction of O_2.[8] Antioxidant can act by scavenging reactive oxygen species (SOD removing O_2), by inhibiting their formation (e.g.by blocking activation of phagocytes), by binding transition metal ions and preventing formation of OH and or decomposition of lipid hydroperoxides, by repairing peroxyl damage (e.g. α-tocopherol reparingperoxyl radicals and so terminating the chain reaction of lipid peroxidation.[9]

Many living species have several antioxidant defense system against oxidative stress induced by reactive oxygen species (ROS). These system include anti oxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), etc. SOD and CAT has been identified to play an important role in life span determination.[10]

Actual aim of *Bheshajasevanakala*(time of administration of medicine) is to provide the fulfillment towards desired action of drug administration in patient in order to pacify the disease condition. The main intension of it to carry a large amount of drug in required time. It is useful to maximize desired effect and lower down the side effects of the drug. It treats the diseases by maintaining the equilibrium of dosha's and dhatu's. It cures the disease by palliative way. Rasayana Tantra is a branch by which one attains longevity, memory, intelligence, freedom from disorders, youthfulness, excellence of luster, excellence of complexion and voice, optimum strength of physique and sense organ, successful words, respectability and brilliance. Rasayana (promotive treatment) means the way for attaining excellence of rasa etc. (dhatus).

It is increasingly being realized that many of today's diseases are due to "oxidative stress" that results from an imbalance between formation and neutralization of free radicals. Free radicals are produced in the body as byproducts of normal metabolism, as a result of exposure to radiation and some environmental pollutants. Because they are highly reactive, they can damage cellular components and are implicated in a variety of diseases. Free radicals are normally neutralized by efficient systems in the body that include the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) and the nutrient-derived antioxidant small molecules (vitamin E, vitamin C, carotenes, flavonoids, glutathione, uric acid, and taurine). In healthy individuals, a delicate balance exists between free radicals and antioxidants.

SOD Finding: One of the crucial antioxidant defenses of the Amalaki is SOD, which are the only enzyme family with activity against superoxide radicals. It catalyzes the dismutation of superoxide radicals ($O^{\cdot}2$) into O2 and H2O2. In the present study the serum SOD level was decreased in group 1 from 11.05 units/ml before treatment to 8.09 units/ml. As compared to control where it is increased from11.31 units/ml to 14.37 units/ml. Thus from the above result it was revealed the antioxidant activity of Amalaki in group 1 is more as compared to control where it was not decreased, thus it is highly significant statistically. The serum SOD level was decreased in group 1 from 11.05 units/ml before treatment to 8.09 units/ml. as compared to group 3 where it is decreased from 10.98 units/ml to 9.77 units/ml. Thus it is highly significant. The serum SOD level was decreased in group 1 from 11.05 units/ml before treatment to 8.09 units/ml. as compared to group 2 where it is decreased from 12.12 units/ml to 9.74 units/ml. Thus it is highly significant. Similarly by comparing other groups with group 1 it was revealed that the anti-oxidant

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activity of Amalaki is highly significant. As per Sajan J et. al., (2009)[11]&Bethke TD et.al.(2010)[12] the rate of drug absorption and peak concentration is greater with morning than other absorption. As stated above in group 1 the healthy individuals were given Amalakichurna at morning time, thus it can be assumed that there may be increased in drug absorption and peak concentration in blood plasma of participants during clinical study. The antioxidant activity is performed by SOD the only enzyme known to use free radical (Super oxide O_2^-) as a substrate. Bhattacharya et.al (1999)[13].It can be interpreted that, our changing lifestyles have meant that certain unhealthy traits have entered our lives, directly or indirectly. These traits are affecting our quality of life adversely such as growing pollution, population, stress and strain, competitive workplace, lack of exercise, sedentary profession, improper eating habits, and emotional insecurity.

Modern science has recently acknowledged the importance of kala and is termed as Chronopharmacology. The drug optimization can be achieved through Chronopharmacology. Chronopharmacology is the Science that deals with the variations in the pharmacological actions of various drugs over a period of 24 hours of the day. The biochemical, physiological and pathological variations of the 24 hour period in humans had been well described in the Ayurvedic texts although the Modern Science was not much aware of it until the 20th Century. The Pharmacokinetics and Pharmacodynamics of a medication and nutrients are directly affected by the endogenous biological rhythm. The effectiveness of many drugs varies depending on the dosage administration time associated with 24 hours biological rhythm under the control of circadian clock.[14]Circadian rhythms are self-sustaining endogenous oscillations occurring in a period of 24 hours. The circadian rhythms are related to the normal sleep-wake cycle. These rhythms are controlled by Suprachiasmatic nuclei (SCN) that are situated in the hypothalamus and the pineal gland. This master clock network regulates the circadian clocks located in cells, tissues and organ-systems. The chronopharmacologic approaches tend to reduce the side effects and to make the drug more bio-available. The conventional homeostatic approach is replaced by the proper study of Chronopharmacology. The Chronopharmacological principle is used in the therapy of Myocardial Infarction, diabetes, hypertension, bronchial asthma, arthritis, hypercholesterolemia etc.[15]

So to increase free radicals only one factor to cause aetiopathologyis not sufficient there are some other factors responsible for producing oxidative stress which lead to increase in free radicals. Other factors may be in the form of interactions along with infection, inflammation, protease/antiprotease imbalance, oxidative stress, environmental pollution and apoptosis. Also, genetic factors and diet can affect the pathogenesis of producing free radicals.

The free radicals scavenging activity of SOD is effective only when it is followed up by increase in the activity of CAT. Since SOD generates hydrogen peroxide as a metabolite, which is more tissue toxic than oxygen radicals and has to be scavenged by CAT. Thus, a concomitant increase in CAT activity is essential if a beneficial effect from increase in SOD activity is to be expected.[16]

V. CONCLUSION

By administrating Amalakichurna as Rasayana to evaluate oushadhasevan kala i.e. time of administration of medicine and its free radicals scavenging activity on healthy individuals the following conclusions were drawn:

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- The dose and duration of Amalaki churna defined was competent enough to act as Rasayana and to scavenge free radicals.
- Amalaki is effective broad-spectrum antioxidants and free radical scavengers, helping to reduce disease and slow down the aging process.
- Primary antioxidants such as Superoxide dismutase and Catalase are first and most important line of defense against highly reactive, potentially destructive oxygen-derived free radicals and Amalaki stimulates these enzymes.
- Evaluation of oushadhasevan kala i.e time of administration of medicine was the main objective of the study which come back with highly significant results, on the basis of Oushadhasevan kala.Amalaki boost weakened antioxidant defenses in Kinchitsuryodayajate kala (morning time of administration)
- Medicine given at the appropriate time will be conducive for the better treatment. The bheshajaSevana kala (time of administration of medicine) is mainly governed by dominance of particular doshawhich is responsible for biological rhythms and is targeted for the treatment. The drug optimization can be achieved by administering the medicine in an appropriate time. For management of Rasayana Karma the ideal bheshajasevanakalais Kinchitasuryodayajateas per clinical and experimental evidence. Bheshajasevanakalais having its own scope and application in the management of diseases. By incorporating proper bheshajasevanakalaone can enhance the bioavailability, target the disease site and relieve symptoms.
- This study revalidates oushadhasevana kala w.s.r. to Amalaki.
- Its revalidation of concepts of Ayurveda.

Hence further studies should be designed considering many representatives of various drugs with different oushadhasevan kala i.e. time of administration of medicine.

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