WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1121-1126. Research Article ISSN 2277– 7105

IN VITRO SCREENING OF FREE RADICAL SCAVENGING ACTIVITY OF SHILAJATU (ASPHALTUM PUNJABINUM) BY LIPID PER OXIDATION METHOD WITH SPECIAL REFERENCE TO RASAYANA KARMA.

Hirekar Sachin Narayanrao[1*], Kharat Ravindra Sahebrao[2], Toshniwal Mahendra Bansilal[3], Kanti V. G.[4],

[1]Designation: Assistant Professor, Department of Rasashastra, CSMSS Ayurved Mahavidyalaya, Kanchanwadi, Aurangabad. 431005.

[2]Associate Professor, Department of Dravyagunavigyan, CSMSS Ayurved Mahavidyalaya, kanchanwadi, Aurangabad. 431005.

[3]Assistant Professor, Department of Sharirkriya, CSMSS Ayurved Mahavidyalaya, Kanchanwadi, Aurangabad. 431005.

[4]Associate professor, Department of Rasashatra and Bhaishjya Kalpana, AVS Mahavidyalaya, Bijapur.

Article Received on
17 Aug 2015,

Revised on 10 Sept 2015,
Accepted on 04 Oct 2015,

***Correspondence for Author**
Hirekar Sachin Narayanrao
Designation: Assistant Professor, Department of Rasashastra, CSMSS Ayurved Mahavidyalaya, Kanchanwadi, Aurangabad. 431005.

ABSTRACT

Maintenance & promotion of positive health is more important than treatment of diseased one. It is possible from Rejuvenative drugs (Rasayana dravyas) which prevents ageing by scavenging free radicals, act as antioxidants & promotes immunity of an individual. Numbers of antioxident drugs have been explained in Ayurvedic texts. A scientific study is need of the hour. To provide evidence-based data, an attempt was made in this regard with “Screening of free radical scavenging activity of Shilajatu”.

KEYWORDS: Free Radicals, Shilajatu, Vitamin C, Lipid Per Oxidation.

INTRODUCTION

Ayurveda does not merely believe in adding the number of years to life but it advocate to add life to each year. This may be one of the reasons

that the first aim of Ayurveda is to preserve and promote the health of healthy persons. To fulfill this aim Ayurveda maintains one separate branch that is Rasayana that is rejuvenating therapy. Rasayana mainly deals with promotion of physical and mental health.

The properly and timely use of Rasayana Therapy promote youthfulness, provides longevity, increases memory & intelligence, complexion, body glow and best physical strength as well as of senses.[1,2]

The term Free radical scavenging activators is being used more by nutritionists and by other health professionals. In general "free radical scavenging activators" are known as "Anti oxidant" which neutralizes free radicals that are generated in the body and prevent damage to the cell proteins, lipids and carbohydrates.[3]

Harmful free radicals play an important role in immune system dysfunction, which is responsible for various diseases like cancer and cardiovascular, arthritis, ulcerative colitis, asthma, allergy, parasitic diseases.[4,5]

Rasayanas are a group of non-toxic herbals as well as mineral drug preparations, which are used to improve the general health by stimulating the body's immunity. Numbers of anti oxidant single drug or herbo-mineral formulations are mentioned in ayurvedic classics. In charaka samhita Rasayana chapter it is mentioned that.[6]

The administration of shilajatu at proper time with proper yogas can cure almost all the curable diseases in this world, And according to rasaratnasamuchaya, shilajatu possess properties like jwarhara (anti pyretic), pandughna (cures anemia),pramehaghna (anti diabetic)and meda chedak (which clears fat).Therefore it is expected to have anti-oxidant activity.

In review of shilajatu it was observed that a vast study was done on shilajatu for its hypoglycemic activity, spermatogenic activity, ovogenic activity and so on.

Hence to provide the scientific data and statistical validation the study was undertaken.

Aims of the Study

1. To assess the free radical scavenging activity of Shilajatu.
2. To provide scientific data for Rasayana (rejuvenation) property of Shilajatu.

MATERIALS AND METHODS

Parameters used for assessment of Free radical scavenging activity:

1. Lipid Peroxidation.

MATERIALS

Drug: a) Shilajatu (standerdised as per API)

b) Vitamin C (Tablet Limcee-550 mg)

METHODOLOGY

Lipid Peroxidation i.e. Thiobarbituric acid reacting substance Assay (TBARS).[7]

Principle

The proteins in serum are precipitated by trichloroacetic acid (TCA) and the mixture is heated with thiobarbituric acid in 2 M sodium sulfate, in a boiling water bath for 30 minutes. The resulting chromogen is extracted with n-butyl alcohol and absorbance of organic phase is determined at 530 nm wavelength. The values are expressed in terms of malondialdehyde nmol/ml as reference standard.

Procedure

Aliquots of 4 ml of homogenate were taken in six different small conical flasks. All the test tubes are added with 6 ml of Potassium sulphate buffer (pH 7.4), 8 ml of 0.15m Potassium chloride. Drug was excluded in first two control groups. In test group three different conical flasks added three concentrations of drug like 1%, 2% and 5%.In standard group Vitamin-C is added. Finally 40µl of carbon tetra chloride (CCl_4) was added except second control. Totally six flasks were incubated at 37°C in incubator. Lipid peroxidation is assessed on 1st day (after 45 min), 2nd day and 4th day. On each day 0.5ml of reaction mixture from the homogenate which is kept for incubation is taken in a test tube and added with 4ml of 10% Trichloroacetic acid (TCA). Contents centrifuged at 4000 rpm for 10 min. 2ml of clear supernatant is taken in a graduated tube. 2 ml of 0.67% Thiobarbituric acid (TBA) is added and heated in boiling water bath for 15 min. Then tubes are cooled, pH is adjusted to 12-12.5.Colour, which is developed stabilized. Absorbance is measured at 540 nm in an UV spectrometer.

Observations and Results

Shilajatu subjected for in-vitro screening of free radical scavenging activity.1%, 2% and 5% of solution of Shilajatu was prepared with distilled water. For the preparation of homogenate

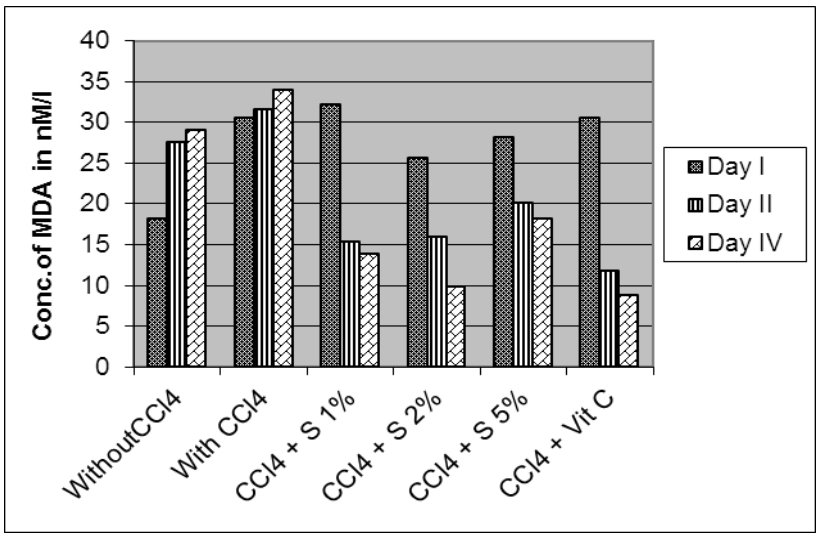
soon after sacrificing the rats liver excised immediately and flushed with normal saline to prevent clots in the tissue.CCl_4 was used to induce oxidative stress as it produces oxidative damage to cells. Two-control group were made to observe normal level of antioxidant enzymes and extent of oxidative stress induced with CCl_4.Different concentrations of Shilajatu were made to find accurate dose. MDA standardization was carried out first to plot the standard graph.End product of Lipid peroxidation i.e MDA reacts with Thiobarbituric acid to give pink colour which is used as marker for lipid peroxidation. Lipid peroxidation and other antioxidant parameters SOD, GSH and CAT assessed on 1st, 2nd and 4th day to observe the time taken by drug for action.

Table No. 1: Shows the concentration of MDA in estimation of lipid peroxidation

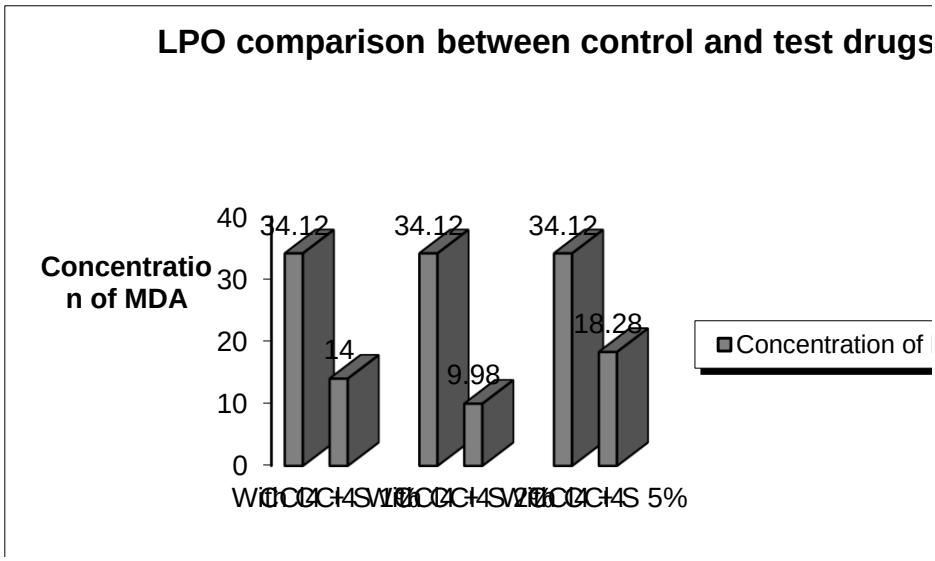
(Concentration of MDA in nM/l)

Sr. No.	Test Sample	Day I	Day II	Day IV
1	WithoutCCl4	18.33	27.6	29.11
2	With CCl4	30.59	31.71	34.12
3	CCl4 + SJT 1%	32.3	15.5	14
4	CCl4 + SJT 2%	25.74	15.97	9.98
5	CCl4 + SJT 5%	28.2	20.14	18.28
6	CCl4 + Vit C	30.6	11.94	8.88

Maximum MDA concentration was decreased in 2% Shilajatu solution



Graph No.1: Showing comparison between all the sample (day wise) in Lipid peroxidation



Graph No.2: **Showing comparison between control and test drug in Lipid peroxidation**

DISCUSSION

Free radical scavenging activity of Shilajatu was assessed on rat liver homogenate. In which oxidative stress was induced with CCl_4. Parameters taken were Lipid peroxidation, which indicates the extent of oxidative stress CCl_4 is a halomethane and a known hepato-toxin. It produces cellular degeneration by producing free radicals. These free radicals can interact with membrane structures there by generating lipid peroxides. Exact mechanism may be by inhibiting mitochondrial respiration i.e inhibition of consumption of O_2 by mitochondria.

In case of lipid peroxidation on first day concentration of MDA was found more in homogenate treated with only CCl_4 i.e 30.59 nM/ ltr. This indicates oxidative stress induced by CCl_4. This was reduced significantly in homogenate treated with Shilajatu 1%, 2% and 5%. On 4th day 2% Shilajatu found more effective in which MDA concentration was reduced to 9.98 nM/ltr than 1% Shilajatu (14 nM/ ltr.) and comparable to standard drug Vit. C (8.88 nM/ltr). When lipid peroxidation results of first, second and fourth day were compared oxidative stress was found to be increased in second and fourth day gradually in all the groups. Even than concentration of MDA in test group was less compared to control (with only oxidative stress), 2% of Shilajatu solution was significant in reducing MDA concentration than 1% (32.3, 15.5, 14), 5% (28.2, 20.14, 18.28) and comparable to standard (30.6, 11.94, 8.88).

CONCLUSION

In this study it is noted that, Shilajatu potentiates the antioxidant activity. The rat liver homogenate treated Shilajatu showed less degree of CCL_4 induced lipid peroxidation. Worldwide debate is on for the use the ayurvedic minerals and metallic preparations. Therefore this study may become more relevant and contribute scientific base. CCl_4 induces oxidative stress by free radical mechanism.In case of lipid peroxidation on first day concentration of MDA was found more in homogenate treated with only CCl_4 ie 30.59 nM/ ltr. This indicates oxidative stress induced by CCl_4.MDA concentration and oxidative stress were reduced significantly in homogenate treated with Shilajatu 1%, 2% and 5%. Significant effect was found with 2% in which MDA concentration was reduced to 9.99nM/ltr and comparable to standard drug vitamin-C (8.80 nM/ltr.) On second and fourth day also Shilajatu reduced concentration of MDA. The effect of 1% & 2% of Shilajatu solution was better than 5%.

REFRENCES

1. Harman D: The free radical theory of ageing: consequences of mitochondrial ageing, 1983; 6: 86-92.
2. Cutler RG. Peroxide – producing potential of tissues: inverse co-relation with longevity of mammalian species. Proc. Natl. Acad. Sci. USA, 1985; 82: 4798-4802.
3. Joe E. Swanson:Antioxidant Nutrients, Cornell Nutrition Expert Overview, Sept 1999; 2.
4. P. Shanthakumari.:Modulation of oxidative stress parameters by treatment with piper betle leaf in streptozotocin induced Diabetic rats: Indian Journal of Pharmc, 2003; 35: 373-78.
5. Patwardhan B.:Indian drugs, 1990; 28(2): 56-63.
6. Bramhanand Triphati:1st chapter, Chikitsa sthana, Charak samhita, Chaukhambha, Surbharti, Varanasi.
7. Kei Satoh:Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method Clin.Chimica Acta, 1978; 90: 37-40.