AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF *RAJATA BHASMA & RAJATA YOGA*

M.Durga Bhavani[1]*, Ch.Sridurga[2]
*[1]M.D Scholar Final year, [2]Associate professor & H.O.D, Dept. of Rasa Shastra & Bhaisajya Kalpana, S.V.Ayurvedic College, Tirupati, Andhra Pradesh, India.

KEYWORDS: Oxidative stress, Diabetes mellitus, *Rajata Yoga*, *Rajata bhasma.*

***Address for correspondence**
Dr M.Durga Bhavani
M.D Scholar Final year,
Associate professor & H.O.D,
Dept. of Rasa Shastra & Bhaisajya Kalpana, S.V.Ayurvedic College,
Tirupati, Andhra Pradesh, India.
Email:
bhavanimutha@gmail.com
Phone no. 9908153898

ABSTRACT

Change in life style has resulted in the increased production of free radicals in the body which not only raise the oxidative stress but also plays an important role in the immune system dysfunction. Now a day, human body is continuously exposed to different types of agents that result in production of reactive species called as 'free radicals'. Diabetes is a group of metabolic diseases characterized by high levels of blood sugar (hyperglycemia). Many evidences from experiments have proved that free radicals and oxidative stress play a major role in complications of Diabetes mellitus like coronary heart disease, Neuropathy, Nephropathy, Retinopathy and stroke. In Ayurveda, the syndrome Diabetes mellitus has been covered under the broad heading of *Prameha*. *Rajata Yoga* is mentioned in *Rasa Tarangini* and indicated in all types of *Prameha*. *Rajata bhasma, Twak churna, Ela churna* and *Patra Churna* are the main ingredients of *Rajata Yoga.* The aim of present study is to evaluate the in vitro anti oxidant activity of *Rajata bhasma & Rajata Yoga*. The method followed to evaluate the anti oxidant activity is 1, 1- diphenyl 2- picrrylhydrazyl (DPPH) radical scavenging assay has been used in the determination of In- vitro antioxidant activity.

Results: Significant Inhibition percentage of *Rajata bhasma & Rajata Yoga* was seen in increasing order of percentage when sample concentration (100µl, 200µl, 300µl, 400µl, 500µl) was increased. *Rajata bhasma* showed 69.86% of inhibition and *Rajata Yoga* showed 80.10% of inhibition in 500 µl sample concentrations respectively.

INTRODUCTION

Prameha as a disease itself is having a peculiar type of *Samprapti*. The *Ahara Vihara* which causes *Kapha Medo Mutra Vrudhi* is the causative factors of *Prameha*[1]. Based on the signs and symptoms, Diabetes Mellitus can be correlated with *Prameha*. Diabetes Mellitus is a group of metabolic disorders that is characterized by elevated levels of glucose in blood and insufficiency in production or action of insulin produced by the pancreas inside the body.[2] Oxidative stress can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug action and toxicity, inflammation and / or addiction. In general, it is called as excess formation or / and insufficient removal of highly reactive molecules such as reactive nitrogen species (RNS), reactive carbon species (RCS) and reactive oxygen species (ROS)[3]. Nowadays, evidences have been reported that support the role of oxidative stress in the pathogenesis of both type 1 and type 2 diabetes.[4] It is believed that in the onset and progression of late diabetic complication, free radicals have got a major role due to their ability to damage lipids, proteins and DNA.[5] To tackle these free radicals our body needs antioxidants. Many herbal and herbo-mineral compound preparations have been screened for their anti oxidant properties but still there is a need for effective antioxidants. *Rajata bhasma* is an important metallic preparation used in the management of *Prameha, Gridhrasi, Nadi sula Unmada* etc[6]. *Rajata Yoga* mentioned in *Rasa Tarangini* is indicated in all types of *Prameha. Rajata bhasma* and *Trijataka churna* are the main

ingredients of *Rajata Yoga.* Hence *Rajata bhasma & Rajata Yoga* were prepared classically and were screened to validate their free radical scavenging activity.

AIMS AND OBJECTIVES

1. To prepare *Rajata Yoga* according to *Rasa Tarangini*.
2. To screen the free radical scavenging activity of *Rajata bhasma* & *Rajata Yoga* by DPPH method.

MATERIAL AND METHODS

Pharmaceutical Study[7]

Samanya Shodhana (General purification) and *Visesha Shodhana* (specific purification) procedures were adopted for *Rajata patra* (silver foils- 50g). After *Visesha Shodhana*, 48g of coarse powder of *Rajata* were obtained. It was subjected to *Marana* (Incineration) with equal quantity of *Kajjali* (Metacinabar-48g) and sufficient quantity of *Kumari Swarasa* (*Aloe vera* juice) as *Bhavana dravya*. Totally 25 *Putas* were given to attain *Rajata bhasma* (90g) which passed all *Bhasma lakshanas* as mentioned in *Rasa* classics i.e., *Rekhapurnatwa, Varitaratwa, Slakshantwa* and *Mrudutwa*. *Rajata bhasma* (45g) was mixed with *Trijataka churna (Twak churna*-300g; *Ela churna* -300g and *Patra churna* – 300g) to form 945g of *Rajata Yoga*.

DPPH radical scavenging activity

The antioxidant activity of *Rajata Yoga* sample was expressed in terms of IC_{50} (micro molar concentration required to inhibit DPPH radical formation by 50%). The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1, 1- diphenyl-1- picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (100, 200, 300, 400 and 500 µg/mL) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 minute incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation:

$$\% \text{ of scavenging} = \frac{(A \text{ control} - A \text{ sample})}{(A \text{ control})} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents expect the test compound) and A sample is the absorbance of the test compound. Tests were carried in triplicate.

Table 1: Showing the volume of samples taken in the procedure

	Blank	Control	A	B	C	D	E
Sample Concentration	-	-	100µl	200µl	300µl	400µl	500µl
Concentration of sample in µg/ml	-	-	10	20	30	40	50
Volume of Methanol	1000µl	1000µl	990µl	980µl	970µl	960µl	950µl
DPPH	-	1ml	1ml	1ml	1ml	1ml	1ml

RESULTS

DPPH is a commercial oxidizing radical which can be reduced by anti-oxidants. The disappearance of the DPPH radical is based on the absorbance at 517nm wavelength which can be monitored by decreased optical density (OD). The inhibition percentage of *Rajata bhasma & Rajata Yoga* is as shown below.

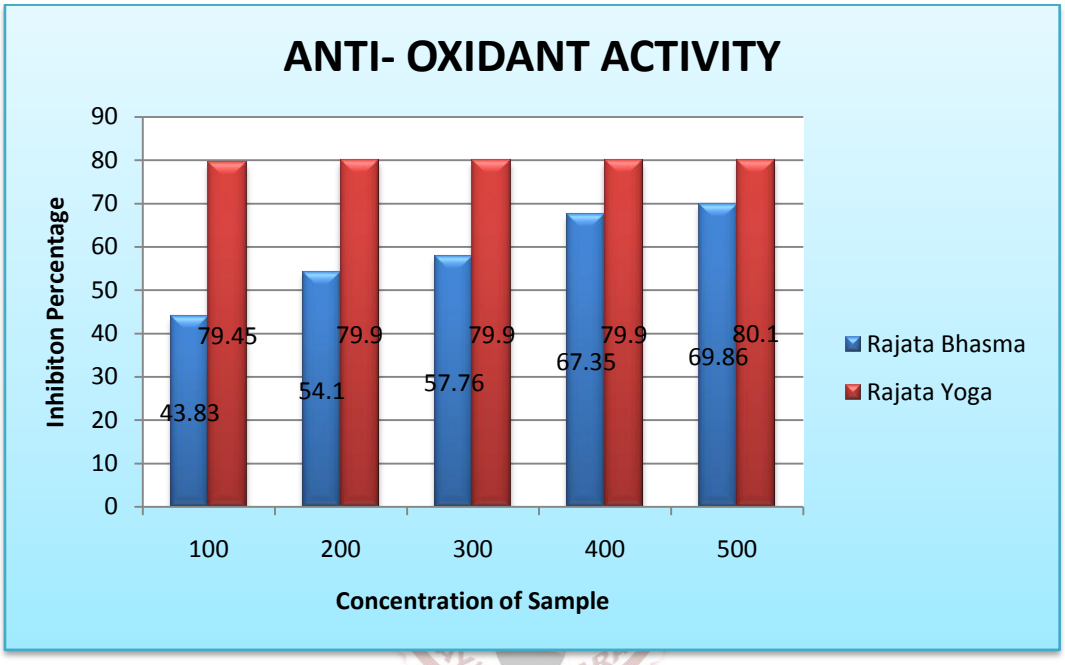
Table 2: Showing the result of Anti- oxidant activity of *Rajata bhasma*

Sample concentration	Sample OD	Inhibition %
A	0.246	43.83
B	0.201	54.10
C	0.185	57.76
D	0.143	67.35
E	0.132	69.86

Table 3: Showing the result of Anti- oxidant activity of *Rajata Yoga*

Sample concentration	Sample OD	Inhibition %
A	0.090	79.45
B	0.088	79.90
C	0.088	79.90
D	0.088	79.90
E	0.087	80.10

Graph 1: Showing the Anti – oxidant activity of *Rajata bhasma & Rajata Yoga*



DISCUSSION

Free radicals are reactive chemical entities that are short lived species containing one or more unpaired electrons. They can induce damage to cells by passing the unpaired electrons resulting in oxidation of cell components and molecules. They are generally very unstable and very much reactive[8]. The term 'anti oxidant' can be labelled for any substance whose availability, even in minute concentrations inhibits or delays the oxidation of a substrate [9]. Antioxidants can be divided as either chain breaking anti-oxidants or preventive anti-oxidants, based on their mechanism of action. Different types of anti-oxidants include Glutathione, Vitamin C & E, Cystine etc. It is believed that oxidative stress plays important role in the development of vascular complications in Diabetes. In- vivo studies support the role of hyperglycemia in the generation of oxidative stress leading to endothelial dysfunction in blood vessels of diabetic patients.[10] Lipids, proteins, DNA damage, Glutathione, catalase and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus.

Lipids

A critical biomarker of oxidative stress is Lipid per oxidation. Experimental studies show that polyunsaturated fatty acids in cell membrane are extremely prone to attack by free radicals due to the presence of multiple bonds. [11]

Proteins

Diabetic hyperglycemia, by the process of free radical production, causes protein glycation and oxidative degeneration. The degree of such protein glycation is estimated by using some biomarkers such as glycated hemoglobin and fructosamine levels.[12]

Glutathione

Diabetes induces alterations in activity of enzymes glutathione peroxidase and glutathione reductase. These enzymes are found in cell that metabolizes peroxide to water and converting glutathione disulfide back into glutathione. Any alteration in their levels will make the cells prone to oxidative stress and hence cell injury [13].

Catalase

Catalase is regulator of hydrogen peroxide metabolism that can, in excess, cause serious damage to lipids, RNA and DNA. In case of catalase deficiency, beta cell of pancreas that contain large amount of mitochondria, undergoes oxidative stress by producing excess ROS that leads to β-cells dysfunction and ultimately diabetes. [14]

Superoxide dismutase (SOD)

Superoxide dismutase provides first line defense against ROS mediated cell injury by catalyzing the proportion of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide. [15]

The results of evaluation of antioxidant activity of *Rajata bhasma* and *Rajata Yoga* indicated significant activity in both the preparations. *Rajata Yoga* showed relatively superior anti oxidant activity when compared to *Rajata Bhasma.* It was proved that highest concentration of linoleic acid in *Cinnamomum zeylanica* is suggestive of its protective mechanism against lipid oxidation[16]. The anti oxidant activity of *Eletteria cardamomum* was due to presence of phenolic compounds like Linalool, Eugenol, Limonene and Spathulenol [17]. The leaves of *Cinnamomum tamala* was proved to show antioxidant activity by containing high contents of antioxidants like phenol, ascorbate and carotenoids [18]. Many research worked proved the anti oxidant activity of green synthesis of Silver nano particles [19]. With the increase in the sample concentrations, the percentage of inhibition of free radicals was also seen increasing. This indicates that the antioxidant activity of both *Rajata Bhasma* and *Rajata Yoga* are dose dependent. Higher anti oxidant activity of *Rajata yoga* may be due to the addition of other herbal ingredients to the *Rajata bhasma*. All the herbal ingredients present in the *Rajata Yoga* have been proved for their antioxidant activity.

CONCLUSION

Oxidative stress has been demonstrated in many studies to participate in the progression of Diabetes and elevation of its complication incidence. From the results obtained from the present study, it can be confirmed that *Rajata bhasma* and *Rajata Yoga* have significant anti oxidant activity. Thus, we can conclude that *Rajata Yoga* may be used more effectively in the management of *Prameha.*

REFERENCES

1. Agnivesa's Charaka Samhita Nidana sthana by R.K.Sharma; Choukambha Sanskrit Series office, Varanasi, Reprint 2009: pp No. 226;
2. Handbook of Diabetes- Ruby Bilous MD, FRCP & et al, Wiley – Blackwell publication; 4th edition: pp no. 2.
3. Sies H, 10985 Oxidative stress, Pubmed; PMID – 9129943; 1997 Mar, 82(2): 291-5.
4. Matrimi, A C, Sanders, R.A, Watkins- Diabetes, Oxidative Stress and antioxidants-A review. Journal of Biochemistry &Molecular Toxicology 17 (1), 24-38.
5. Ayelpola, O.R, Brooks, N L, 2014. Oxidative stress and Diabetic complications: The Role of Antioxidant Vitamin and Flavanoids. (http://dx.doi.org/10.5772/57282).
6. Sri Vagbhataacharya, Rasaratna Samuchaya by Indradev Tripati, Choukambha Sanskrit Samsthan; Varanasi. Reprint 2013; p. 99.
7. M. Durga Bhavani, Ch.Sridurga – Parmaceutical Standardisation of Rajata Yoga. International Journal of Ayurveda and Pharma Research. 2017; 5(8): 28-36.
8. Bansal, A.K., Bilaspuri, G.S., 2011. Impacts of oxidative stress and antioxidants on semen functions; Veterinary Medicine Intrnational Volume 2011, ID 686137, 7 pages.
9. Somogyi, Anik´ o et al - Antioxidant measurements; Pubmed- PMID – 1739589; 2007.Apr. 28(4); R41–55.
10. Ceriello, A., 2006. Oxidative stress and diabetes-associated complications. Pubmed-PMID – 16627383; 2006, 12 (l), 60–62.
11. DA Butterfiel et al, -. Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants N-tert-butyl alpha-phenylnitrone and vitamin E. Pubmed; PMID: 9928452; 1998 Nov. 20; 854, 448–62.
12. Nishigaki, I., Hagihara, M., Tsunekawa, H., Maseki, M., Yagi, K- Lipid peroxide levels of serum lipoprotein fractions of diabetic patients; Biochemical Medicine; volume 25, Issue 3, June 1981, pp. no.373–378.
13. Maritim, A.C., Sanders, R.A., Watkins - Diabetes, oxidative stress and antioxidants: A review. Journal of Biochemistry Molecular Toxicology; 17 (1), 24–38.
14. Jamieson, Dana, 1986. The relation of free radical production to hyperoxia. Ann. Rev. Physiol. 48, 703–719.
15. Tiwari, B.K. et al, Markers of oxidative stress during diabetes mellitus; Journal of Biomarkers; volume 2013, ID 378790, 8 pages.
16. J. Mancini-Filho et al - Antioxidant activity of Cinnamom (Cinnamomum zeylanica, Breyne)

extracts; Pubmed January 1999; PMID: 10077878.

17. Farhad Sharafati et al - In vitro anti bacterial and anti oxidant properties of Eletteria cardamomum Maton extract and its effects, incorporated with chitosan, on storage time of lamb meat; Veterinarski Arhvi 87(3), 301-315, 2015.
18. Salma Akter & et al -In vitro antioxidant and cytotoxic activity of ethanolic extract of Cinnamomum tamala leaves; International Journal of Pharma sciences and Research. Volume 6 No.3 March 2015; ISSN: 0975-9492.
19. Thilagavathi & et al - Anti oxidant activity and synthesis of silver nanoparticles using the leaf extract of Limonia acidissima; International Journnal of Pharma and Bio Sciences: 2016 Oct; 7(4) (B) 201-205.

Cite this article as:
M.Durga Bhavani, Ch.Sridurga. Evaluation of Antioxidant Activity of Rajata Bhasma & Rajata Yoga. AYUSHDHARA, 2016;4(3):1183-1187.