WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 6, 639-650. Research Article ISSN 2277– 7105

ROLE OF *BHARGAVAPROKTHA RASAYANA* TREATMENT IN REDUCING THE SYSTEMIC INFLAMMATION & DNA DAMAGE IN PEOPLES EXPOSED TO FLY ASH FROM THERMAL POWER PLANT

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Article Received on
04 March 2019,

Revised on 24 March 2019,
Accepted on 15 April 2019,

DOI: 10.20959/wjpr20196-14760

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ABSTRACT

Objective: To investigate whether the *Bhargavaproktha rasayana* treatment is effective in reducing the systemic inflammation in apparently healthy individuals exposed to fly ash from thermal power plant. **Design:** Study Type - Interventional; Allocation - Randomized; Endpoint Classification - Efficacy Study; Intervention Model - Parallel Assignment; Masking: double blind; Primary Purpose – Preventive treatment. **Setting:** 60 healthy volunteers residing within the 3 km diameter of National thermal power plant Nandikuru, Udupi district and 30 healthy volunteers outside the 10 km diameter of thermal power plant during the period September 2017 to January 2019. **Participants:** 60 healthy volunteers were randomly assigned 1:1 to oral administration of *Bharagavaproktha rasayana* (n=30) or placebo (n=30). The randomization sequence was done by computer generated permuted block randomization and is concealed using sealed sequentially numbered packets. There was no intervention in 30 healthy volunteers, but blood samples taken to assess base line values of primary and secondary

outcome measures. **Main outcome measures**: Primary outcome measures – TNF-α, Secondary outcome measures – Lipid profiles, blood urea, serum creatinine, SGOT, SGPT, glucose, protein and albumin. **Results**: The initial value of TNF-α in *Bhargavaproktha Rasayana* group at base line was 20.28 (±SE 2.60) and which reduced to 13.18 (±SE 1.57) after the 48 days of *rasayana* treatment. This reduction was statistically significant with p=0.0144. **Conclusion**: *Bhargavaproktha rasayana* treatment is effective in reducing the systemic inflammation there by helpful in preventing the development of chronic health complications.

KEYWORDS: *Bharavaproktha rasayana*, Systemic inflammation, DNA damage, fly ash, thermal power plant.

INTRODUCTION

Ayurvedic Rasayanas are a unique group of formulations which chiefly designed to promote health in the healthy, thereby enhancing longevity and maintaining youthfulness. These are widely used in Ayurveda clinical practice both as general adaptogenics as well as in some diseases, especially chronic degenerative diseases. *Rasayana* is a treatment which enhances the *vyadhi kshamatva* of an individual.[1] Aim of the *rasayana* is to reduce the occurrence of any disease, maintenance and improvement of functioning capacity of *dosha* and *dhatu*. As per the disease in literature particular *rasayana* are elaborated termed as *vyadhihara rasayana*.[2] These vyadhihara rasayanas are important for the reconditioning the body against different variety of diseases.[3] These *rasayana* will work on the initial pathology of a disease ie *Khavaigunya* and *srotodusti*. *Rasayana* has its maximum effect if administered after the *kosta shuddi*.[4]

In literature many *rasayana* were explained for *Pranavaha Sroto vikara*, one among is *Bharagaproktha Rasayana*. It has major role in the management of different respiratory diseases like that of *Kasa, Shwasa*, and also beneficial in enhancement of *Bala*.[5] The mode of action of rasayanas need to be studied. Modern techniques, especially in molecular biology, provide highly sensitive tools to evaluate the effect of pharmacological agents, including rasayanas, at cellular level.

The stability of the genome is of crucial importance in human health and yet the DNA molecule is prone to spontaneous loss of bases, and damage from exogenous and endogenous sources – with potentially mutagenic consequences. The various causes & mechanisms of

DNA damage give rise to a perplexing array of DNA lesions which are harmful in general to the human genome and may lead to development of cancer.

The main emissions from coal combustion at thermal power plants are carbon monoxide, nitrogen oxides, sulphur oxides, chlorofluorocarbons, and air- borne inorganic particles such as fly ash, soot, and other trace gas species which are known to cause DNA damage and play role in development of diseases.[6] TNF-α is a cell signalling protein (cytokine) involved in systemic inflammation and is critically involved in the induction of systemic DNA damage.[7] TNF-α induces the enhancement of reactive oxygen intermediates (ROI) leakage from the mitochondrial respiratory chain and that this directly or indirectly leads to DNA damage and various events subsequent to DNA damage, such as impairment of DNA repair, may also be involved in the development of TNF-α cytotoxicity.[8] Many intracellular processes, such as DNA fragmentation, adenosine diphosphate (ADP) ribosylation, phospholipase activation, oxidative stress, an increase in cytosolic Ca^{2+}, and induction of endogenous nucleases are possibly involved in TNF-α induced cell lysis.[9] The literature surveys clearly suggest that elevated level TNF-α, in plasma has role in the systemic inflammation and in the DNA damage.

METHODS

Study design: Randomized placebo controlled study with blinding of volunteers and assessors. All volunteers are recruited to the after taking written consent.

Volunteers, Randomization and Treatment: Healthy volunteers of both sexes between the age group of 30 – 50 years, residing within 3 km diameter of national thermal power plant, Nandikuru, Udupi and spending minimum of 10 hours per day in this particular area were eligible for the study. Volunteers suffering from chronic diseases like diabetes, kidney diseases, cancer, thyroid dysfunction and any other chronic illness and on medications were excluded from the study. Volunteers having the habit of cigarette smoking, tobacco chewing, alcohol consumption of every day were also excluded from the study. Eligible volunteers recruited for the study and randomly allocated to *Bharagavaproktha rasayana* and placebo in 1:1 ratio. The 50 gm of *Bhargavaproktha rasayana* and placebo in *leha* form of identical colour and appearance sealed in a packet. The volunteers of the *Bhargavaproktha rasayana* group given a total of 48 packets having same number. Similarly volunteers of the placebo group given relevant placebo. Volunteers of both groups are asked to consume a dose of 50 gm every morning on empty stomach with warm water for 48 days. Before starting of the

treatment i.e. one day prior *kosta shudhi* procedures were performed on volunteers of both groups by giving *Trivit leha* of 10 gm on empty stomach, early morning before the food. Volunteers were advised to restrict themselves for bland diet for one day i.e. on the day of *kosta shudhi* procedure. All the volunteers tolerated the dose very well and recorded no side effects.

Volunteers of both sexes between the age group of 30 -50 years, residing outside the 10 km diameter of thermal power plant were recruited to control group. Volunteers not suffering from chronic diseases like diabetes, kidney diseases, cancer, thyroid dysfunction and any other chronic illness and not on medications, and not having the habit of cigarette smoking, tobacco chewing and alcohol consumption of every day were recruited to control group.

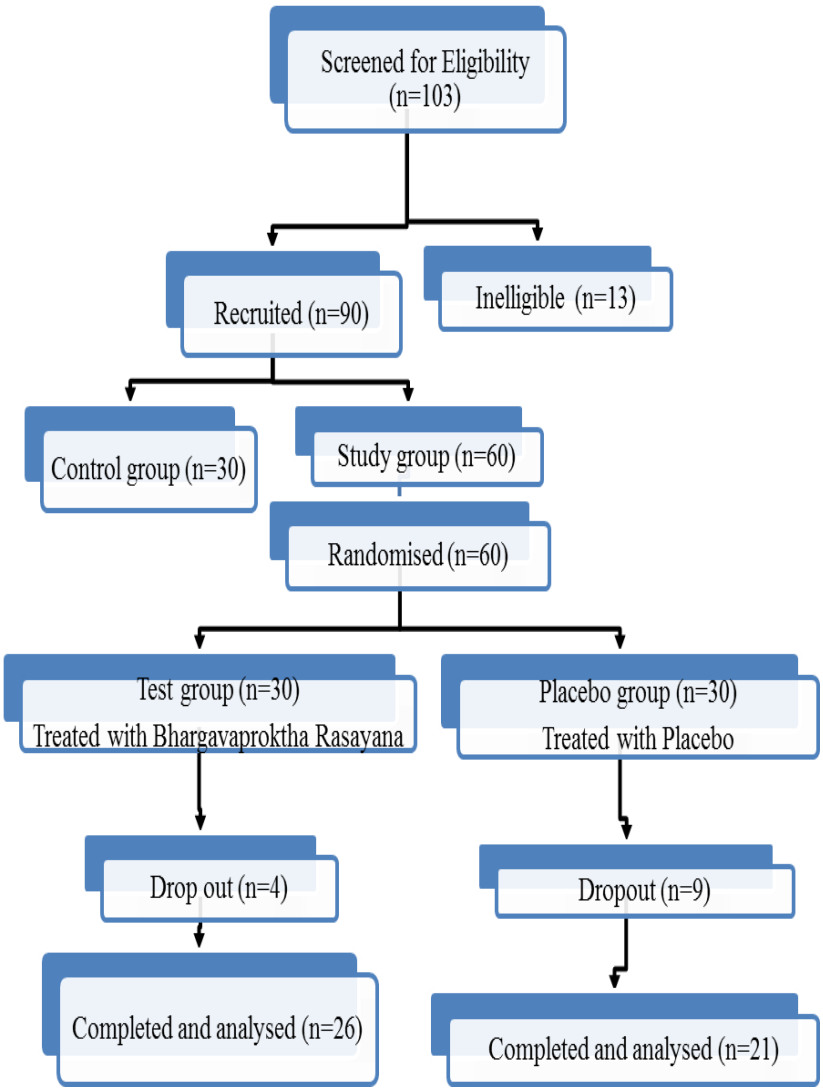

Fig 1: Volunteers flow diagram.

Bhargavaproktha rasayana: Based on Ayurvedic School of Pharmaceutical practices, the ingrediants of *Bhargavaproktha rasayana* are as follows

- *Pradhana dravya* (Main drug) – *Amalaki* constitutes the prime ingredient in the formulation.
- The *Samsadhana dravyas* (Processing drug) – This set of ingredient is used to process the main drug, *Amalaki*. Thirty six herbs are included in this group. It comprises of *Bilva, Arani, Shyonaka, Gambhari, Patala, Bala, Shalparni, Prishnaparni, Mashaparni, Pippali, Gokshura, Brihati, Kantakari, Shringi, Mudgaparni, Bhumyalaki, Draksha, Jeevanti, Pushkharamula, Agaru, Abhaya, Amrita, Riddhi, Jeevaka, Rishabhaka, Shati, Musta, Punarnava, Meda, Ela, Utpala, Chandana, Vidarikanda, Vasa, Kakoli* and *Kakanasa*.
- *Yamaka dravyas* (Lipid media) – This group is mainly used to treat the main constituent, *Amalaki* fruits.
- *Samvahaka dravyas* (Preservative drug) – This act as carriers or vehicle for the main and supportive ingredients. Since they preserve clinical efficacy of main drug, this set of ingredients could be referred to as preservative drugs. *Matsyandika* (crystal sugar) used in the formulation.
- *Prakshepaka dravyas* (Flavoring drug) – This also termed as balancing drugs, this group includes *madhu, tuga, pippali, tvak, ela, patra* and *keshara*.

Procedures and outcome

Primary Outcome Measure is the level of TNF-α after 48 days of administration of *Bhargavaproktha rasayana* or placebo. The value of TNF-α is expressed in pg/L and was measured by ELISA technique. TNF-α was measured at baseline and after 48 days of treatment, and compared statistically.

Haemoglobin, lipid profile, SGOT, SGPT, total protein, albumin, serum creatinine, blood urea and blood glucose were the secondary outcome measures in both the arms. These parameters are recorded as mg%, g/dl, UL as per the protocol and were measured at baseline and are compared to the value at 48 days. The day of randomization and the first dose of *leha* defined as day 0 and the day 48 is the time point of primary outcome of the clinical trial.

Statistical analysis

Volunteers were recruited from September 2017 till November 2018 and total of 90 volunteers, of 30 each in three groups were recruited. The data obtained were analyzed using

Graph pad InStat (version 3.05). Within the groups the data were tested reporting mean median minimum and maximum values, standard deviation as well as standard error. The continuous variables of primary outcome measure of TNF-α and the secondary outcome measures of serum creatinine, blood urea, SGOT, SGPT, protein, albumin, lipid profile, blood glucose and haemoglobin were compared for any change following treatment with in the groups by adapting the paired t test and between the groups by the method of unpaired t test. Base line value of primary outcome measure TNF-α was compared with control with *rasayana* group and control with placebo group.

RESULTS

Sixty volunteers included in the trial were recruited during the period from September 2017 to November 2018 from the area within 3 km area of National thermal power plant Nandikuru, Padubidri, Udupi district for study group. Thirty volunteers recruited during the same above mentioned period from the area in and around SDM Ayurveda college & hospital kuthpady, Udupi district for control group. Sixty volunteers were randomly allocated into *Bhargavaproktha rasayana* and placebo group. These volunteers received the medication as per the allocated group. Twenty six (87%) volunteers completed the intervention in the *Bhargavaproktha rasayana* group and twenty one (70%) volunteers in placebo group.

Mean age of the volunteers was 43.87 years (SD±5.104) in *Bhargavaproktha rasayana* group, 43.90 years (SD±4.908) in placebo group and 44.07 years (SD±4.417) in control group (Table 01). Among the 60 volunteers in the study group (*Bhargavaproktha rasayana* & Placebo) 58% were women, 68% were married, 83% were Hindu, 43% had high school education, 32% were household, 67% were of lower middle class socioeconomic state, 73% had mixed diet, 75% of volunteers recorded medium physical activity (Table 02).

Table 01: Age of 90 volunteers involved in the project.

Profile	Group	Mean	±SD	±SE	Max	Min	Median
AGE	*Rasayana*	43.87	5.104	0.9319	50	30	44.00
	Placebo	43.90	4.908	0.8962	50	33	43.50
	Control	44.07	4.417	0.8065	50	35	44.50

Table 02: Volunteers demographics in the study group (*Rasayana* & Placebo).

Sl no	Profile	Category	Volunteers					
			Rasayana		Placebo		Total	
			No	%	No	%	No	%
1	Gender	Male	12	40	13	43	25	42
		Female	18	60	17	57	35	58
2	Marital status	Single	07	23	06	20	13	22
		Married	20	67	21	70	41	68
		Widowed	03	10	02	07	05	08
		Divorcee	00	00	01	03	01	02
3	Religion	Hindu	26	87	24	80	50	83
		Muslim	03	10	04	13	07	12
		Christian	01	03	02	07	03	05
		Jain	00	00	00	00	00	00
		others	00	00	00	00	00	00
4	Educational status	Postgraduate	00	00	00	00	00	00
		Graduate	04	13	03	10	07	12
		Intermediate	10	33	11	37	21	35
		High school	14	47	12	40	26	43
		Primary school	02	07	04	13	06	10
		Illiterate	00	00	00	00	00	00
5	Occupation	Professionals	00	00	00	00	00	00
		Farmers	08	27	09	30	17	29
		Office work	04	13	04	13	08	13
		Skilled worker	05	17	03	10	08	13
		Household	10	33	09	30	19	32
		Unskilled worker	03	10	05	17	08	13
		Unemployed	00	00	00	00	00	00
6	Socio-economic class	Upper	00	00	00	00	00	00
		Upper middle	00	00	00	00	00	00
		Lower middle	19	63	21	70	40	67
		Upper lower	09	30	09	30	18	30
		Lower	02	07	00	00	02	03
7	Diet	lacto vegetarian	05	17	04	13	09	15
		lacto-ovo vegetarian	04	13	03	10	07	12
		Mixed diet	21	70	23	77	44	73
8	Physical Activity	Inactive	00	00	00	00	00	00
		Low	04	13	05	17	09	15
		Medium	23	77	22	73	45	75
		High	03	10	03	10	06	10

Comparison of baseline values between the groups:

The mean TNF-α, at base line was 19.47 pg/ml (SE ±1.72) in study group (*Bhargavaproktha rasayana* & placebo) and the same in control group was 12.18 pg/ml (SE ±1.04). When these values were compared between the study & control groups at base line by unpaired t test the change observed was statistically significant with p = 0.0053 (Table 03) indicating that there

was systemic inflammation and systemic DNA damage in people residing within 3km diameter area of thermal power plant compare to people residing outside the 10 km diameter of thermal power plant.

Table 03: Comparison of baseline values of the Primary outcomes measure (TNF-α) in Control and Study groups.

Test (pg/ml)	Group (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha	Control (30)	12.18	5.70	1.04	34.6	6.0	10.10	2.857	0.0053
	Study (60)	19.47	13.34	1.72	72.0	6.9	15.55		

Treatment effect on TNF-α and comparison between the groups

Table 04 represents the results of primary outcome measure of TNF-α in the placebo group. The initial mean value of TNF-α in placebo group at base line was 18.66 (±SE 2.29) and which reduced to 17.76 (±SE 2.46) after the intervention. This improvement was statistically non significant with p =0.2098. On the other hand, the table 05 represents the results of the *Bhargavaproktha rasayana* group. The base line mean value of TNF-α was 20.28 (±SE 2.60) in *rasayana* group and reduced to 13.18 (±SE 1.57) after the intervention. This improvement was statistically significant with p=0.0144. Thus definite improvement was recorded in *Bhargavaproktha rasayana* group.

Table 04: Comparison of primary outcome measure (TNF- α) before and after treatment in the placebo (study) group.

Test (pg/ml)	Placebo Group (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha	BT (30)	18.66	12.55	2.29	72	6.9	16.8	1.296	0.2098
	AT (21)	17.76	11.26	2.46	58	7.2	15.0		

Table 05: Comparison of primary outcome measure (TNF- α) before and after treatment in the *Rasayana* (study) group.

Test (pg/ml)	*Rasayana* Group (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha	BT (30)	20.28	14.26	2.60	64.0	7.2	15.10	2.631	0.0144
	AT (26)	13.18	8.01	1.57	34.8	5.0	9.95		

Further statistical analysis of TNF-α between the groups (Table 06) i.e. Placebo after the treatment compared with base line value of Control group still shows statistically (p=0.0144)

significant difference, thus indicating that there was no reduction in systemic inflammation and DNA damage after the intervention with placebo. Statistical analysis shows no significant difference (Table 07) in TNF-α in the *Bhargavaproktha rasayana* group after the intervention and base line value in the control group (p=0.5993), indicating after the treatment systemic inflammation and DNA damage is considerably reduced, thus coming to the level of healthy volunteers residing outside the 10 km diameter area of thermal power plant.

Table 06: Comparison of Primary outcome measure (TNF- α) of the Placebo (Study) group after the treatment with baseline value of Control group.

Test	Group (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha (pg/ml)	Placebo AT (21)	17.76	11.26	2.46	58	7.2	15.0	2.089	0.0462
	Control (30)	12.18	5.70	1.04	34.6	6.0	10.10		

Table 07: Comparison of Primary outcome measure (TNF- α) of the *rasayana* group after the treatment with base line value of Control group.

Test	Group (N)	Mean	± SD	±SE	Max	Min	Median	T test	P Value
TNF alpha (pg/ml)	*Rasayana* AT (26)	13.18	8.01	1.57	34.8	5.0	9.95	0.5293	0.5993
	Control (30)	12.18	5.70	1.04	34.6	6.0	10.10		

Treatment effect on Secondary outcome measures and comparison between the groups

In the secondary outcome measures statistically significant change was observed in Cholesterol, LDL with reduction in the mean value after the treatment of *rasayana*. HDL, albumin and haemoglobin also shows statistically significant change with increase in the mean value after the *rasayana* treatment (table 08). When the response was compared between the groups by the method of unpaired t test the change observed between the groups was statistically non significant indicating the improvement may be due chance factor.

Table 08: Secondary outcome parameters -Treatment effect and comparison between the groups.

Parameters	Group	Mean Value		BT-AT	Within group*		Between Group**	
		BT	AT		T	P	T	P
Cholesterol (mg/dl)	Rasayana	211.86	201.30	10.56	2.447	0.0218	0.01146	0.9909
	Placebo	209.70	201.19	8.51	0.9025	0.3718		
Triglycerides (mg/dl)	Rasayana	144.63	131.65	12.98	1.175	0.2512	0.08668	0.9313
	Placebo	167.10	142.85	24.25	1.010	0.3246		
HDL (mg/dl)	Rasayana	52.40	58.96	-6.56	5.210	0.0001	1.469	0.1491
	Placebo	54.90	55.09	-0.19	2.299	0.0324		
LDL (mg/dl)	Rasayana	127.20	116.57	10.63	2.186	0.0384	0.2786	0.7819
	Placebo	125.53	119.09	6.44	1.991	0.0603		
SGOT (U/L)	Rasayana	27.67	26.04	1.63	1.490	0.1487	1.973	0.0548
	Placebo	31.40	30.0	1.4	0.1807	0.8584		
SGPT (U/L)	Rasayana	26.03	25.81	0.22	1.856	0.0752	2.674	0.0105
	Placebo	33.2	32.14	1.06	0.8729	0.3931		
Total Protein	Rasayana	7.05	7.25	-0.20	2.057	0.0503	0.6010	0.5510
	Placebo	7.35	7.15	0.20	0.4886	0.6304		
Albumin	Rasayana	4.18	4.40	-0.22	2.437	0.0223	0.5417	0.5908
	Placebo	4.25	4.32	-0.07	1.769	0.0922		
Blood Urea	Rasayana	23.87	23.35	0.52	0.9626	0.3450	3.096	0.0034
	Placebo	27.73	27.86	-0.13	0.9550	0.3510		
Creatinine	Rasayana	0.84	0.87	-0.03	1.370	0.1830	1.336	0.1900
	Placebo	0.89	0.91	-0.02	1.793	0.0881		
Glucose	Rasayana	98.47	100.04	1.57	0.665	0.5119	0.4472	0.6570
	Placebo	101.70	101.95	-0.25	1.422	0.1705		
Haemoglobin	Rasayana	101.70	101.95	-0.25	1.422	0.1705	0.7783	0.4405
	Placebo	12.36	12.25	0.11	0.4886	0.6304		
*Paired T test, **Unpaired T test								

DISCUSSION

In this randomized double blind placebo controlled clinical study 30 volunteers were treated with *Bhargavaproktha rasayana* for 48 days and was found to be effective in reducing TNF-α levels suggesting there is decrease in systemic inflammation & systemic DNA damage in comparison to the placebo treatment. Most of the individuals were recorded the reduction in the TNF-α levels after the *rasayana* treatment. The reduction in the systemic inflammation, support the applicability of *rasayana* treatment in general improvement of health and youthfulness. Also considerable positive change was observed in some of the lipid parameters, albumin and haemoglobin. At the same time it revealed there is no observable change in the renal and liver parameters.

Strengths and limitations of this study

Double blind RCT is considered as the gold standard in deriving clinical conclusion. Needless to say this double blind randomized design, with necessary concealment of allocation, blinding therapists and the assessors of outcome measures rules out the any possibility of bias in the result interpretation. The use of very sensitive primary outcome measures of TNF-α by ELISA gives clear picture of the response to the treatment with no any ambiguity of subjective errors in the evaluation. This need to be considered in further research designs in this regard.

CONCLUSIONS

Bhargavaproktha rasayana is effective in reducing systemic inflammation and DNA damage in individuals exposed to fly ash from thermal power plant. *Rasayana* treatment especially *Bhargavaproktha rasayana* will help in improving the health status of individuals residing near the thermal power plant exposed to fly ash.

What is already known on this topic?

Rasayanas are widely used in *Ayurveda* clinical practice both as general adaptogenics as well as in some diseases, especially chronic degenerative diseases. *Bharagaproktha Rasayana* has major role in the management of different respiratory diseases like that of *Kasa*, *Shwasa*, and also beneficial in enhancement of *Bala*. TNF-α is a cell signalling protein (cytokine) involved in systemic inflammation and is critically involved in the induction of systemic DNA damage. TNF-α induces the enhancement of reactive oxygen intermediates (ROI) leakage from the mitochondrial respiratory chain and that this directly or indirectly leads to DNA damage.

What this study adds?

Gives data based evidence that treatment with *Bhargavaproktha rasayana* improves the general health conditions of public exposed air pollution especially fly ash from thermal power plants by decreasing systemic inflammation and systemic DNA damage.

Ethical approval

This study was approved by the Institutional research ethics committee.

Trial registration: RGU:R&D:Res.Wing:2014-15 DATED:13-03-2015.

Acknowledgment: Advance Research Wing, Rajeev Gandhi University of Health Sciences, Bangalore for sponsoring the project.

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