Original Research Article

HEALING ROLE OF *GUDUCHI* [*Tinospora cordifolia* (Willd.) Miers] AND *AMALAKI* (*Emblica officinalis* Gaertn.) CAPSULES IN PREMATURE AGING DUE TO STRESS: A COMPARATIVE OPEN CLINICAL TRIAL

ABSTRACT

Aims: To find out the comparative effects of the capsules prepared from the drugs *Guduchi* [*Tinospora cordifolia* (Willd.) Miers] and *Amalaki* (*Emblica officinalis* Gaertn.) in cases of premature aging due to stress.

Study design: Comparative open clinical trial

Place and Duration of Study: IPD and OPD of National Institute of Ayurveda and Seth Soorajmal Bombawala Hospital, Jaipur, Rajasthan, India, between June 2016 and April 2017.

Methodology: We included 30 patients (14 men, 16 women; age range 25-60 years) with premature aging due to stress, without any other acute or serious systemic disorders. They were randomly divided in three groups with 10 patients in each. Group A- was treated with capsule of *Guduchi* 1 gm/day, Group B– capsule of *Amalaki* 1 gm/day and Group C- was treated with 500mg capsules of *Guduchi* and *Amalaki* separately once daily for 3 months. Visual Analogue Scale and Stress were assessed by Hamilton Anxiety Rating Scale (HARS). Objective parameters like CBC, CRP, FBS, Blood Urea, creatinine, SGOT, SGPT and Serum cholesterol were also analysed.

Results: Marked effect of therapy was observed in group C, where the significant changes *($p<0.05$)* were observed on subjective parameters like Dizziness, Constipation, Aching Muscles, Sleep abnormality, Loss of appetite, Fatigue, Generalized Weakness. In HARS scale significant changes (*$p<0.01$*) were found on Anxious Mood and Intellectual power, along with on Tension, Fears, Insomnia, Depressed Mood and Gastrointestinal Symptoms statistically significant (*$p<0.05$*) changes were also observed. Similarly on objective parameters like Hb%, Eosinophils and TPLC, significant effect (*$p<0.05$*) of therapy was observed. Along with these marked significant (*$p<0.01$*) effect was observed in improvement of Neutrophil, Lymphocyte, TRBC, TPLC and PCV. But In ANOVA Test for Intergroup Comparison no significant changes (*$p>0.05$*) were found except on the parameters like abnormality in sleep, FBS and TLC where *p* value was found to be statistically significant *($p<0.05$)*.

Conclusion: Both the test drugs, stem of *Guduchi* and dried fruit rinds of *Amalaki* were found to be significantly effective in premature ageing due to stress, but the effect was quantitatively better in Group C (*Guduchi* and *Amalaki*). However these findings need further validation in large scale study.

Keywords: *Premature aging, Stress, Amalaki, Guduchi, Herbal, Ayurveda*

1. INTRODUCTION

Ayurveda is not only a system of medicine rather a manner of life. According to *Acharya Charaka* life is the blend of body, senses, mind and reincarnating soul[1]. The age is a factor dependent on *kala pramana vishesha* i.e. quantum of time duration[2]. *Acharya Sushruta* has mentioned a group of naturally occurring diseases named *svabhava bala roga*, which includes *kshudha* (hunger)*, pipasa* (thirst)*, nidra* (sleep)*, jara* (aging) and *mrityu* (death)*.* The last phase of life span has been referred as *jara* (aging) which is described as a natural and inevitable processes as well as natural disease.[3] It is a continuous process which begins with conception and end with death. In ancient *Ayurveda* classics*, Jara* has been categorized in two Headings- *kalaja* (irreversible) and *akalaja* (reversible)*. Kalaja* is a natural

phenomenon, which stems from inherited potential, but *akalaja* (reversible) is premature ageing which may be triggered due to physical and mental stress.[4] According to latest health report, stress is now becoming more accepted as being crucially related to our total physical, mental and spiritual health. Various stresses lead to disturbance in the homeostasis of both the body and mind by vitiating *manasdosha* (mental humor), *shariradosha* (body humor) and *agni* (conflagration of heartiness) [5].Thus stressful environment and disturbance in *manasika bhava* has adversely affected the healthy life style and that gives rise to the symptoms of aging before the time [6]. A revolution of life style, changing family structure, economic crisis and social problems, are major stress inducers affecting developing countries. Various stressors that lead to disturbance in *manasabhava* (emotions), described in *Ayurveda,* are root cause of many diseases including premature ageing[7]. The *kama* (lust), *krodha* (anger), *lobha* (greed), *moha* (delusion), *irsha* (jealousy), *shoka* (grief), *chinta* (anxiety), fear (*bhaya*) etc. are different *manasabhava*[8].The nerve-racking atmosphere and annoyance of these *manasabhava* adversely affect the health which ultimately contributes to premature ageing.

The drug *Guduchi* botanically identified as *Tinospora cordifolia* (Willd.) Miers belongs to Menispermaceae family and the drug *Amalaki* botanically identified as *Emblica officinalis* Gaertn. belongs to family Euphobiaceae are widely available throughout tropical and sub-tropical region of India[9], also frequently used by the *Ayurveda* physicians in the condition of premature ageing. Both the drugs are mentioned *as vrishya* (aphrodisiac), *medhya* (promote intellectual properties of brain), *valya* (enhance vitality), *rasayana* (rejuventive and immunomodulator) and *vayahsthapana* (anti aging) in ancient *Ayurveda* texts.[10,11] Various pharmacological studies also find their significant effects as immunomodulator, adaptogenic, anti oxidant, anti stress, anti microbial and anti allergic.[12,13]. On this background the present study was under taken to find out comparative effects of the capsules prepared from the drug *Guduchi* and *Amalaki* in the premature aging due to stress.

2. MATERIAL AND METHODS

2.1. Collection of Drugs: Stem of *Guduchi* [*Tinospora cordifolia* (Willd.) Miers] and Fruits of *Amalaki* (*Emblica officinalis* Gaertn.) were collected from the natural sources and the materials were identified and authenticated by the Department of Botany, Rajasthan University, with voucher specimen no. RUBL211612 (*Guduchi*) and RUBL211616 (*Amalaki*).

2.2. Method of Preparation of Trial Drugs: *Ghana* (concentrated decoction) was prepared separately from both the selected plant parts i.e stem of *Guduchi* and fruits of *Amalaki*, as per the method mentioned in *Sharangadhara Samhita*[14]. Then both the decoctions were again boiled until water content was evaporated to make in to powdered form. Then the 500 mg of powder were filled in each gelatine capsule (with the help of Automatic Capsule filling instrument) separately for both the samples.

2.3. Ethical Clearance: Present clinical trial was done after getting the ethical approval from Institutional Ethical Committee of National Institute of Ayurveda, Jaipur with approval no. IEA/ACA/2015/29.

2.4. Selection of study subjects: 30 Patients having the sign and symptoms of premature aging due to stress were selected from OPD and IPD of National Institute of Ayurveda and Seth Soorajmal Bombawala Hospital, Jaipur, Rajasthan, India. They were randomly divided in three groups with 10 patients in each group.

2.4.1. Inclusion criteria:

- Patients having the signs and symptoms of premature aging due to stress were selected.
- Patients of either sex with the age group between 25- 60 years were included.

2.4.2. Exclusion criteria:

- Patients suffering from chronic diseases like severe Hypertension, IHD, COPD, DM, Cancer, hepatic and renal insufficiency and psychotic disorders like depression, schizophrenia.
- Patients suffering from any other acute or serious illness.

2.4.3. Withdrawal criteria:

- Patients who may develop any adverse drug reaction due to the trial drugs.
- Non complaints of the patients.

- Any Serious Intercurrent Illness

2.5. Method of study: The study was carried out as per International conference of Harmonization-Good Clinical Practices Guidelines (ICH-GCP). Written informed consent was taken on prescribed Proforma from each patient willing to participate before the start of study. They were briefed about merits and demerits of research plan before taking the consent. Patients were free to withdraw from the study at any time without giving any reason. A detailed Proforma was prepared incorporating *Ayurveda* as well as modern points. Observations were made according to the standard *Ayurveda* parameters selected and findings were recorded in well-designed Proforma.

2.5.1. Grouping and administration of drug: 30 clinically diagnosed and registered patients of premature aging due to stress were selected based on inclusion and exclusion criteria. They were divided randomly into 3 groups, each group was 10 patients.
Group A- 500 mg soft Gelatine Capsules of *Guduchi* were given for 3 months, 1 gm/ day in two divided doses.
Group B–500 mg soft Gelatine Capsules of *Amalaki* were given for 3 months, 1 gm/ day in two divided doses.
Group C- 500 mg/day capsule of *Guduchi* and 500 mg/day capsule *Amalaki* were given for 3 months, once daily in the form of soft Gelatine capsule.

2.5.2 Criteria for assessment: The assessments of the patients were done based on subjective as well as objective criteria during the course of trial treatment. The final assessment was done on the basis of all the parameters and by comparing the laboratory investigations before and after the treatment.

2.5.3. Subjective criteria: Visual Analogue Scale and Stress were assessed by Hamilton Anxiety Rating Scale (HARS) [15]

2.5.4. Objective criteria: CBC, CRP, F.B.S, RFT- Blood Urea, Serum creatinine, SGOT, SGPT, Serum cholesterol.

2.5.5. Statistical analysis: Graph Pad prism-7 software was used for analysis of the data obtained from the study. For Non parametric Data Wilcoxon matched-pairs signed ranks test and for intergroup comparison Kruskal- Wallis multiple comparison tests (Dunn's multiple comparison) was used. While for Parametric Data Paired 't' Test and for intergroup comparison ANOVA test was used. Subjective parameters were assessed by the research team as per established grading system.

3. RESULTS AND DISCUSSION

3.1. Effect on Subjective parameters:

Statistically significant results were observed in parameters like Dizziness, Constipation, Aching Muscles, Sleep abnormality, Loss of appetite, Fatigue and Generalized Weakness in group A (Table-1), group B (Table-4) and group C (Table-7). Maximum percentage of relief was observed in group C in all these parameters (Table-1, 4, 7 and Graph-1).

3.2. Effect of Therapy on HARS:

In HARS scale, statistically significant results were observed on the parameters like Anxious Mood, Tension, Fears, Intellectual and Gastrointestinal Symptoms: in group A (Table-2), group B (Table-5) and group C (Table-8) where as in parameters like Respiratory Symptoms, Behaviour at interview, Genitourinary symptoms, Autonomic Symptoms, Somatic (Sensory deformity) no statistically significant changes were observed. Here also maximum response was observed in group C in comparison to group A and B. In the parameters like Insomnia and Depressed Mood, Maximum percentage of relief was observed in group B and group C and in Somatic (Muscular deformity) maximum percentage of relief was observed in group B (Table-2, 5, 8 and Graph-2).

3.3. Effect on Objective parameters:

On objective parameters like Hb% and FBS statistically significant results were observed in group A (Table-3), group B (Table-6) and group C (Table-9). Maximum percentage of relief was observed in group A. On others parameters like Blood Urea and serum SGOT maximum percentage of relief was observed in group B. Whereas in group A shows maximum percentage of relief on the parameters SGPT and serum cholesterol and on Serum Creatinine maximum percentage of relief was observed in group C. Although all these changes were found to be statistically insignificant (*p>0.05*) (Table-3, 6, 9 and Graph-3).

But In ANOVA Test for Intergroup Comparison no significant changes (*p>0.05*) were found except on the parameters like abnormality in sleep, FBS and TLC where *p* value was found to be statistically significant *(p<0.05)* (Table-10, 11, 12 and Graph-1, 2, 3).

3.4. Discussion on probable mode of action of drug:

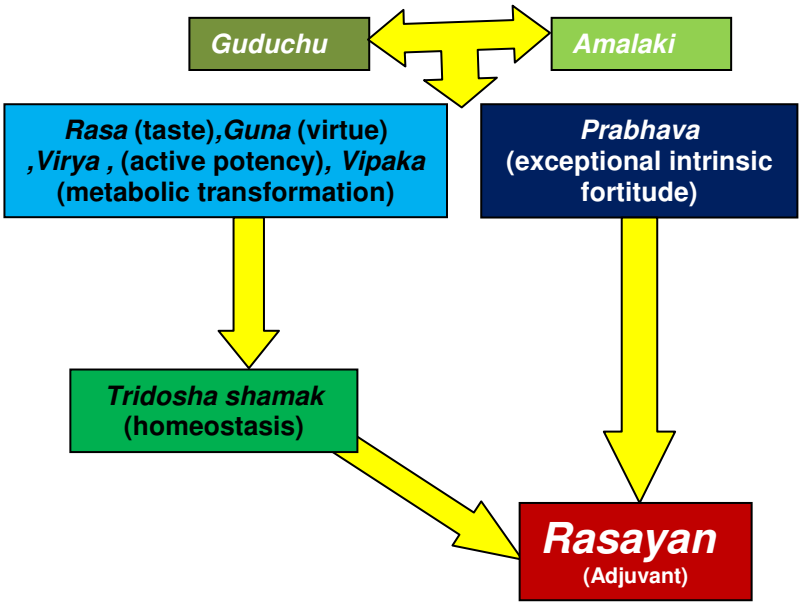
As per the fundamental principle of *Ayurveda* pharmacology, a *dravya* (drug) performs *karma* (certain actions) in the body by virtue of its properties i.e. *guna* (vertue)*, rasa* (taste)*, virya* (active potency)*, vipaka* (metabolic transformation) and *prabhava* (exceptional intrinsic fortitude) which exist in it in a state of co-inherence [16]. The uniformity of proto-elements of the drug on one hand and the proto-elemental constituents of the body on the other hand form the basis of the principles of *samanya vishesha* (egalitarianism and discrepancy)[17]. These principles imply that the predominant proto-elements of the drug will increase similar proto-elements in the body and the dissimilar will decrease the proto-elements. The actions of a drug are intimately related to its chemical structure in the form of preponderance of one or two proto-elements in them. Ancient scholars have advised to use *rasayana* for longevity of life *(ayuanuvruti*, *vayasthapana*) and for achieving the optimum effect of normal function of *tridosha* (physiological humors)[18]*.* The drug *Guduchi* possess *guru, snigdha guna* (heavy and unctuous virtue)*, tikta, kashaya rasa* (bitter and astringent taste) *madhura vipaka* (sweet metabolic transformation)*, ushna virya* (hot active potency)[19].The qualities of *guru* and *snigdha* are nutritive in nature, being similar in quality to *rasadhatu* it enhances and strengthens *rasadhatu* establishing solid grounding for the six remaining *dhatu* (basic physiological structure of body). Being *bitter taste* it pacifies *pittadosha*, while *astringent* as a secondary taste it also balances *kapha*. *Vata* is balanced by the *Guru* and *snigdha* qualities that counter the dry and light qualities of *Vata*. It's hot potency not only stimulates but also correct digestive fire, and digest *amadosha* (undigested food materials)[20]. The presence of the two *Guna, guru* and *snigdha* and the post-digestive action as *madhura vipaka* indicates that the action of the drug is more anabolic rather than catabolic in nature and from an energetic perspective it counters the catabolic nature of aging.

According to *Ayurveda, Amalaki* balances all three *dosha.* While *Amalaki* is unusual in that it contains five out of the six tastes recognized by ancient *Ayurveda* sages*,* it is most important to recognize the effects of the *"virya",* or potency, and "*Vipaka*", or post-digestive effect. Considered in this light, *Amalaki* is particularly helpful in reducing *Pitta* due to its *shita guna* (arctic virtue). It also balances both *Pitta* and *Vata* by virtue of its sweet taste. The *kapha* is balanced primarily due to its *ruksh guna* (seared virtue)[21]. It act as a *rasayana* (rejuvenate) to promote longevity and traditionally to increase *dipanapachana* (digestion and metabolism), *raktaprasadana* (purify the blood), *romasanjana* (stimulate hair growth), *jivaniya* (enliven the body) and *medhya* (enhance intellect)[22]. It ultimately brings out best quality of *dhatu* and slows down the ageing process by generating new cells, antioxidant, anti-atherosclerotic, immunomodulation, free radical scavenging activity, anti-hepatotoxic, adrenergic potentiating, etc[23].

Mental health also plays a vital role in health, disease and premature ageing. *Acharya Charaka* has mentioned that psychological factors cause bodily disorders and vice versa. Again *Charaka* mentioned that keeping body and mind under control, following moral code of conducts and living spiritual life would itself bring the *rasayan* effects and prevent ageing[24]. Both the test drug *Guduchi* and *Amalaki* have effect on subjective parameters and on parameters of HARS either given single or in combination. While normalising the physiology of the body by pacifying *tridosh*, correcting digestion and metabolism and nourishing the *sapta dhatu* in proper way, both drugs release *medhya* effect which correct mental health and stress condition of patient. In an experimental study on *Guduchi* it is proved to have antistress and adaptogenic activity[25]. An ethanolic extract of the roots of *Tinospora cordifolia* normalized stress-induced biochemical changes in norepinephrine Anti-stress activity[26,27].*Guduchi* has been claimed to possess

anti-stress activity[28,29]. Ehanolic extract of *Embelica officinalis* has significant antistress and adaptogenic activity against variety of biochemical and physiological perturbations [30]. It is also claimed as memory enhancing, antioxidant and anti-cholinesterase activity. It may be useful for the treatment of cognitive impairments induced by cholinergic dysfunction[31]. In other similar clinical study on *Amlakyadi rasayana*, significant improvement was reported in *medha* and anxiety scale[32]. On the basis of above facts it can be inferred that *Guduchi* and *Amalaki* both drugs having having *medhya, rasayana* and *vayahsthapana* property. These may be the reasons both drugs provide satisfactory results either single use or in combination (Flow chart-1, 2).

Flow chart-1, *Rasayana* effect of *Guduchi* and *Amalaki:*



Flow chart-2 *Rasayana* effect of *Guduchi* and *Amalaki* to prevent premature aging:

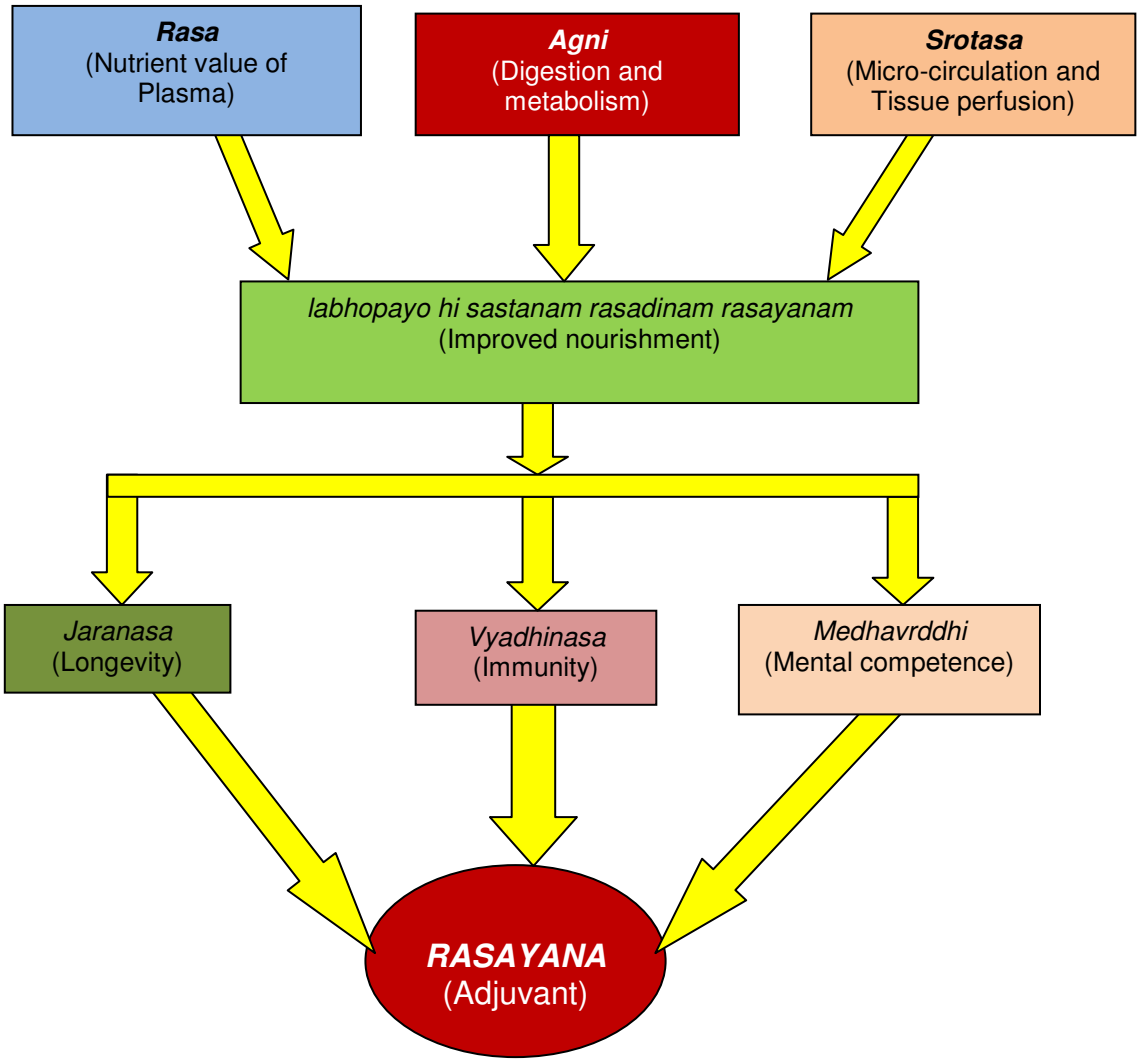


Table 1. effect of therapy on subjective parameters in group A:

Group A	BT	AT	Diff	% of Relief	SD	SEM	(-w)	P value	Sig
Dizziness	1.2	0.3	0.9	75	0.994	0.314	21	0.0313	S
Constipation	0.5	0.3	0.2	40	0.421	0.1333	3	0.5	NS
Aching Muscles	1.3	0.7	0.6	46.15	0.516	0.163	21	0.0313	S
Joint Pain	1.2	0.7	0.5	41.66	0.527	0.166	15	0.0625	NS
Joint Stiffness	1.1	0.6	0.5	45.45	0.527	0.166	15	0.0625	NS

Sleep Abnormality	1.6	1.1	0.5	31.25	0.527	0.166	15	0.0625	NS
Loss of appetite	2.2	1.2	1	45.45	0.471	0.1490	45	0.0039	S
Fatigue	2.7	1.6	1.1	40.74	0.737	0.233	36	0.0078	S
Gen. Weakness	2.5	1.1	1.4	56	0.516	0.163	55	0.0029	S

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 2. effect of therapy on HARS subjective parameters in group A:

GROUP A	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**(-W)**	**P value**	**Sig**
Anxious Mood	2.2	1.3	0.9	40.90	0.56	0.17	36	0.0078	S
Tension	2.8	1.6	1.2	42.85	0.42	0.13	55	0.002	S
Fears	2	1.1	0.9	45	0.87	0.27	21	0.0313	S
Insomnia	1.7	1.3	0.4	23.52	0.51	0.16	10	0.213	NS
Intellectual	1.7	0.8	0.9	52.94	0.31	0.1	45	0.0039	S
Depressed Mood	1.4	0.8	0.6	42.85	0.51	0.16	21	0.0313	S
Somatic muscular	1.2	0.9	0.3	25	0.48	0.15	6	0.2500	NS
Somatic (Sensory)	0.7	0.3	0.4	57.14	0.69	0.22	10	0.125	NS
CVS	0	0	0	0	0	0	0		
Respiratory Symptoms	0.5	0.4	0.1	20	0.31	0.1	1	>0.9999	NS
Gastrointestinal Symptoms	0.6	0.3	0.3	50	0.48	0.15	1	>0.9999	NS
Behaviour at interview	0.6	0.3	0.3	50	0.48	0.15	15	0.0625	NS
Genitourinary symptoms	0.6	0.4	0.2	33.33	0.42	0.13	1	>0.9999	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 3. effect of therapy on objective parameters in Group A:

Group A	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**P value**	**Sig**
HB	13.2	13.64	0.74	5.61	3.708	1.173	0.0207	S

TLC	5540	5760	220	3.97	293.636	92.856	0.0420	S
Neutrophil	55	57.1	2.1	3.82	3.604	1.140	0.0985	NS
Lymphocytes	35.6	38.9	3.3	9.27	2.830	0.895	0.0050	S
Eosinophils	4.2	2.8	1.4	33.33	1.647	0.521	0.0248	S
Monocytes	4.9	5.3	0.4	8.16	1.075	0.340	0.2695	NS
Basophils	0	0	0	0.00	0.000	0.000	0.0000	NS
TRBC	4.83	5.075	0.24	5.07	0.495	0.156	0.1517	NS
TPLC	2.013	2.074	0.06	3.03	0.449	0.142	0.6773	NS
PCV	43.5	42.44	1.05	2.43	2.025	0.640	0.1339	NS
MCV	91.5	90.15	1.38	1.51	2.907	0.919	0.1662	NS
MCH	27.54	26.809	0.731	2.65	1.121	0.355	0.0693	NS
MCHC	30.46	30.117	0.343	1.13	0.556	0.176	0.0830	NS
CRP	0	0	0	0.00	0.000	0.000	0.0000	NS
FBS	96	87.4	8.6	8.96	11.443	3.618	0.0415	S
Blood Urea	32.3	31.4	0.9	2.79	1.912	0.605	0.1708	NS
SR. Creatinine	0.93	0.9	0.03	3.23	0.106	0.033	0.3938	NS
SGOT	41	38.5	2.5	6.10	3.100	0.980	0.0312	NS
SGPT	28.1	27	1.1	3.91	2.283	0.722	0.1619	NS
Sr. Cholesterol	176.5	168.3	8.2	4.65	14.793	4.678	0.1135	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 4. effect of therapy on subjective parameters in group B

Group B	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**(-W)**	**P value**	**Sig**
Dizziness	0.5	0.1	0.4	80	0.516	0.163	21	0.031	S
Constipation	0.7	0.3	0.4	57.14	0.516	0.163	10	0.125	NS
Aching Muscles	3.3	0	2.1	63.63	2.024	0.640	21	0.0313	S
Joint Pain	4.5	3.9	0.6	13.33	0.699	0.221	15	0.0625	NS

Joint Stiffness	2.1	1.8	0.3	14.28	0.483	0.152	6	0.25	NS
sleep abnormality	2.3	1.9	0.4	17.39	1.264	0.4	1	>0.9999	NS
Loss of appetite	2.7	1.5	1.2	44.44	1.229	0.388	21	0.0313	S
Fatigue	2.8	1.3	1.5	53.57	1.840	0.582	21	0.0313	S
Gen. Weakness	4.2	3.5	0.7	16.66	0.674	0.213	21	0.0313	S

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 5. effect of therapy on HARS subjective parameters in Group B

Group B	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**(-W)**	**P value**	**Sig**
Anxious Mood	1.6	1	0.6	37.5	0.51	0.16	21	0.0313	S
Tension	1.4	0.7	0.7	50	0.67	0.21	21	0.0313	S
Fears	1.3	1	0.3	23.07	0.48	0.15	6	0.25	NS
Insomnia	1.4	0.7	0.7	50	0.67	0.21	21	0.0313	S
Intellectual	1.5	0.7	0.8	53.33	0.78	0.24	21	0.0313	S
Depressed Mood	1.3	0.8	0.5	38.46	0.52	0.16	15	0.0625	NS
Somatic (Muscular)	1.2	0.8	0.4	33.33	0.51	0.16	10	0.125	NS
Respiratory Symptoms	0.6	0.3	0.3	50	0.48	0.15	6	0.25	NS
Gastrointestinal Symptoms	0.8	0.4	0.4	50	0.51	0.16	3	0.5	S

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 6. effect of therapy on Objective parameters in Group B

Group B	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**P value**	**Sig**
HB	12.595	12.792	0.197	1.56	0.208	0.066	0.0152	S
TLC	6220	5960	260	4.18	411.501	130.128	0.0768	NS
Neutrophil	62.3	60.7	1.6	2.57	2.914	0.921	0.1165	NS
Lymphocytes	29	26.8	2.2	7.59	3.155	0.998	0.0549	NS

Eosinophils	4.2	3.4	0.8	19.05	1.317	0.416	0.0868	NS
Monocytes	4.5	4	0.5	11.11	0.850	0.269	0.0957	NS
Basophils	0	0	0	0.00	0.000	0.000	0.0000	NS
TRBC	4.463	4.535	0.072	1.61	0.412	0.130	0.5938	NS
TPLC	1.911	1.93	0.019	0.99	0.149	0.047	0.6962	NS
PCV	41.49	41.414	0.076	0.18	0.521	0.165	0.6556	NS
MCV	93.17	92.97	0.2	0.21	0.320	0.101	0.0793	NS
MCH	28.49	28.113	0.377	1.32	0.642	0.203	0.0964	NS
MCHC	30.49	29.925	0.565	1.85	0.865	0.274	0.0690	NS
CRP	0	0	0	0.00	0.000	0.000	0.0000	NS
FBS	98.7	96.7	2	2.03	3.162	1.000	0.0766	NS
Blood Urea	30.7	28.6	2.1	6.84	3.107	0.983	0.0613	NS
SR. Creatinine	0.98	0.84	0.14	14.29	0.201	0.064	0.0552	NS
SGOT	44.9	39.8	5.1	11.36	7.781	2.461	0.0681	NS
SGPT	32.1	32.9	0.8	2.49	4.077	1.289	0.5503	NS
Sr.Cholesterol	177.1	169	8.1	4.57	9.171	2.900	0.0209	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 7.effect of therapy on subjective parameters in Group C

Group C	**BT**	**AT**	**Diff**	**% of Relief**	**SD**	**SEM**	**(-w)**	**P value**	**Sig**
Dizziness	1.6	0.3	1.3	81.25	1.337	0.422	21	0.0313	S
Constipation	1.3	0.3	1	76.92	1.054	0.333	21	0.0313	S
Aching Muscles	2.5	1	1.5	60	1.649	0.521	21	0.0313	S
Joint Pain	2.5	2.1	0.4	16	0.516	0.163	10	0.125	NS
Joint Stiffness	2.2	1.9	0.3	13.63	0.483	0.152	6	0.25	NS
Sleep abnormality	2.7	0.7	2	74.07	1.943	0.614	21	0.0313	S

Loss of appetite	2.9	0.9	2	68.96	2.160	0.683	21	0.0313	S
Fatigue	2.3	1	1.3	56.52	1.159	0.366	21	0.0313	S
Gen. Weakness	2.3	1.1	1.2	52.17	1.135	0.359	21	0.0313	S

Table 8. effect of therapy on HARS subjective parameters in group C

Group C	BT	AT	Diff	% of Relief	SD	SEM	(-W)	P value	Sig
Anxious Mood	1.4	0.6	0.8	57.14	0.42	0.13	36	0.0078	S
Tension	1.1	0.4	0.7	63.63	0.48	0.15	28	0.0156	S
Fears	1.2	0.6	0.6	50	0.51	0.16	21	0.0313	S
Insomnia	1.6	0.8	0.8	50	0.91	0.29	21	0.0313	S
Intellectual	1	0.2	0.8	80	0.42	0.13	36	0.0078	S
Depressed Mood	1.6	0.9	0.7	43.75	0.67	0.21	21	0.0313	S
Respiratory Symptoms	1.2	0.9	0.3	25	0.48	0.15	6	0.2500	NS
Gastrointestinal Symptoms	1.4	0.7	0.7	50	0.48	0.15	28	0.0156	S
Autonomic Symptoms	1	0.8	0.2	20	0.42	0.13	3	0.5000	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 9.effect of therapy on objective parameters in Group C

Group C	BT	AT	Diff	% of Relief	SD	SEM	P value	Sig
HB	12.15	12.71	0.56	4.61	0.631	0.200	0.0205	S
TLC	6100	6150	50	0.82	271.825	85.959	0.5751	NS
Neutrophil	57.4	54.7	2.7	4.70	1.567	0.496	0.0004	S
Lymphocytes	35.3	33.6	1.7	4.82	1.160	0.367	0.0012	S
Eosinophils	2.6	2.2	0.4	15.38	0.516	0.163	0.0368	S
Monocytes	4.7	4.2	0.5	10.64	0.850	0.269	0.0957	NS

Basophils	0	0	0	0.00	0.000	0.000	0.0000	NS
TRBC	4.679	4.479	0.2	4.27	0.166	0.053	0.0042	S
TPLC	2.304	2.16	0.144	6.25	0.196	0.062	0.0449	S
PCV	41.31	40.974	0.336	0.81	0.235	0.074	0.0014	S
MCV	88.28	87.98	0.3	0.34	0.226	0.071	0.0023	S
MCH	26.85	26.76	0.09	0.34	0.197	0.062	0.1823	NS
MCHC	30.37	30.36	0.01	0.03	0.396	0.125	0.9380	NS
CRP	0	0	0	0.00	0.000	0.000	0.0000	NS
FBS	93.8	93.3	0.5	0.53	1.269	0.401	0.2443	NS
Blood Urea	32.5	32	0.5	1.54	1.509	0.477	0.3221	NS
SR. Creatinene	1	0.85	0.15	15.00	0.222	0.070	0.0617	NS
SGOT	39.7	38.4	1.3	3.27	3.368	1.065	0.2533	NS
SGPT	28.2	27.7	0.5	1.77	1.179	0.373	0.2126	NS
Sr. Cholesterol	166.3	164.3	2	1.20	4.163	1.317	0.1631	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 10.analysis of variance (ANOVA) Test on subjective parameters for Intergroup Comparison:

Inter Group Comparison	**Group A**	**Group B**	**Group C**	**P value**	**Sig**
Dizziness	0.4	0.4	1.3	0.2301	NS
Constipation	0.4	0.4	1	0.1050	NS
Aching Muscles	2.1	2.1	1.5	0.3352	NS
Joint Pain	0.6	0.6	0.4	0.8170	NS
Joint Stiffness	0.3	0.3	0.3	0.5741	NS
Sleep abnormality	0.4	0.4	2	0.0481	S
Loss of appetite	1.2	1.2	2	0.7768	NS
Fatigue	1.5	1.5	1.3	0.9497	NS

Gen. Weakness	0.7	0.7	1.2	0.1478	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

Table 11 - Analysis of variance (ANOVA) Test on HARS subjective parameters for Intergroup Comparison:

Inter Group Comparison	Group A	Group B	Group C	P value	Sig
Anxious Mood	0.9	0.6	0.8	0.4221	NS
Tension	1.2	0.7	0.7	0.0749	NS
Fears	0.9	0.3	0.6	0.1975	NS
Insomnia	0.4	0.7	0.8	0.4969	NS
Intellectual	0.9	0.8	0.8	0.8235	NS
Depressed Mood	0.6	0.5	0.7	0.7967	NS
Somatic (Muscular)	0.3	0.4	-	0.0962	NS
Somatic (Sensory)	0.4	-	-	-	-
Respiratory Symptoms	0.1	0.3	0.3	0.4865	NS
Gastrointestinal Symptoms	0.3	0.4	0.7	0.1858	NS
Autonomic Symptoms	-	-	0.2	-	-
Behaviour at interview	0.3	-	-	-	-
Genitourinary symptoms	0.2	-	-	-	-

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

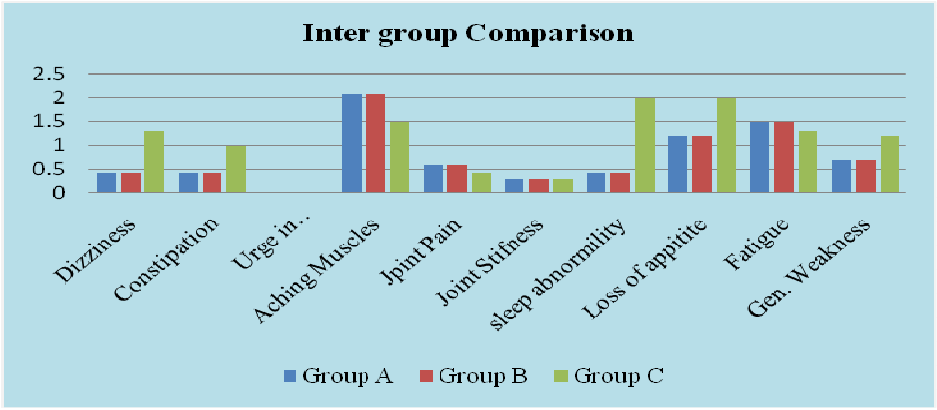
Table 12. analysis of variance (ANOVA) Test on objective parameters for intergroup comparison

Intergroup Comparison	Group A	Group B	Group C	P value	Sig
HB	0.74	0.197	0.56	0.3989	NS
TLC	220	260	50	0.0107	S
Neutrophil	2.1	1.6	2.7	0.9583	NS
Lymphocytes	3.3	2.2	1.7	0.4756	NS
Eosinophils	1.4	0.8	0.4	0.4134	NS

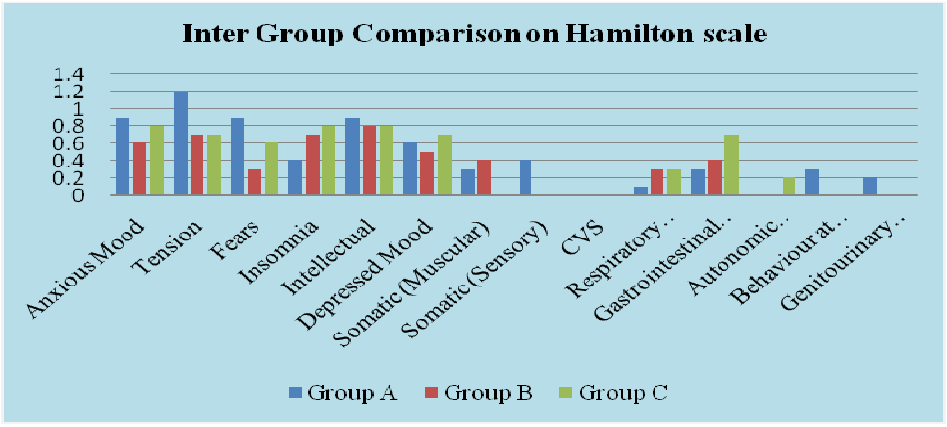
Monocytes	0.4	0.5	0.5	0.0856	NS
Basophils	0	0	0	-	NS
TRBC	0.245	0.072	0.2	0.7483	NS
TPLC	0.061	0.019	0.144	0.6732	NS
PCV	1.055	0.076	0.336	0.1943	NS
MCV	1.385	0.2	0.3	0.2399	NS
MCH	0.731	0.377	0.09	0.1830	NS
MCHC	0.343	0.565	0.01	0.456	NS
CRP	0	0	0	-	NS
FBS	8.6	2	0.5	0.0323	S
Blood Urea	0.9	2.1	0.5	0.0743	NS
SR. Creatinine	0.03	0.14	0.15	0.2850	NS
SGOT	2.5	5.1	1.3	0.1581	NS
SGPT	1.1	0.8	0.5	0.3114	NS
Sr. Cholesterol	8.2	8.1	2	0.3223	NS

* BT-mean of before treatment, AT-mean of after treatment, Diff.-mean difference, SD-standard deviation, SEM-Standard Error of Mean, Sig.-significance level.

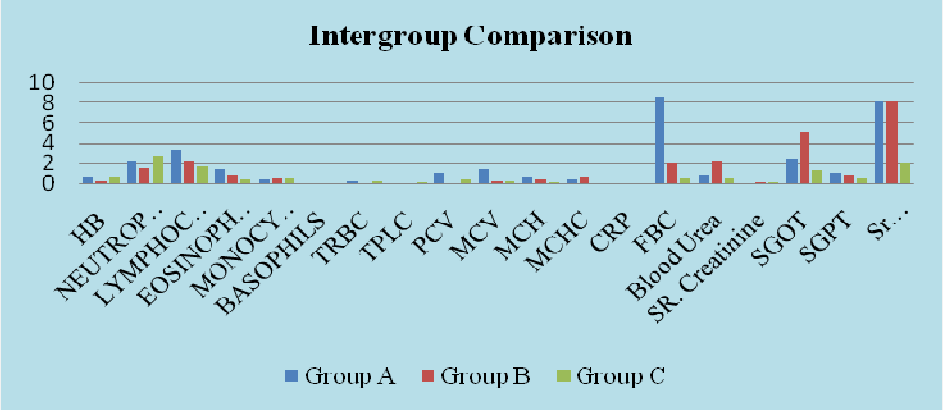
Graph 1. Analysis of variance (ANOVA) Test for Intergroup Comparison on subjective parameters:



Graph 2.comperative effects of therapy in inter group comparison on Hamilton scale:



Graph 3. comperative effects of drugs in inter group comparison on objective parameters:



4. CONCLUSION

Both the test drug, stem of *Guduchi* and Dried fruit rinds of *Amalaki* were found to have significant effect in delaying premature ageing due to stress. But in comparison to other groups the effect was better in Group C (*Guduchi* and *Amalaki*). The trial drugs were tolerated well in the study population as no ADR was observed during the trial duration. The study team suggests that since *Guduchi* and *Amalaki* were found to be effective in delaying premature ageing due to stress, but considering the small size of the trial population, the trial should further be extended to larger sample size and for longer trial duration to draw more conclusive evidence.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this manuscript. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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ABBREVIATIONS

HB-Haemoglobin; TLC-Total Leukocyte Count; TRBC-Total Red Blood Cell Count; TPLC-Total Platelet Count; PCV- Packed Cell Volume; MCV- Mean corpuscular volume; MCH- Mean Cell Hemoglobin;

MCHC- Mean Corpuscular Haemoglobin Concentration; CRP- C-Reactive Protein (CRP); FBS- Fasting Blood Sugar; SGOT- Serum Glutamic-Oxaloacetic Transaminase, It is also known as AST, or Aspartate Aminotransferase; SGPT- Serum Glutamic Pyruvic Transaminase (SGPT), It is also known as Alanine Aminotransferase (ALT).

APPENDIX