Asian Journal of Psychiatry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Asian Journal of Psychiatry

journal homepage: www.elsevier.com/locate/ajp

Evaluation of the influence of ayurvedic formulation (Ayushman-15) on psychopathology, heart rate variability and stress hormonal level in major depression (Vishada)

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 21 July 2014
Accepted 27 July 2014
Available online xxx

Keywords:
Ayurveda
Vishada
Major depressive disorder
HRV
Ayushman
Cortisol

ABSTRACT

Introduction: Ayurveda (Indian-complimentary and alternative medicine) is still most sought after in India and has promising potential in management of Vishada [major depressive disorder (MDD)]. But, systematic research is lacking. In this study we evaluated of influence of ayurvedic treatment (Panchakarma and Ayushman-15) on psychopathology, heart rate variability (HRV) and endocrinal parameters in patients with major depression.

Methods: 81 drug naive patients diagnosed as Vishada by ayurvedic physician and MDD according to DSM IV-TR were given ayurvedic Virechana module (therapeutic purgation) and were randomized into two groups. Patients in group A ($n = 41$) received Ayushman-15A while group B ($n = 40$) received Ayushman-15B for two months and Shirodhara (forehead-oil pouring therapy). Patients were assessed with Hamilton Depression Rating Scale (HDRS), Montgomery Asberg Depression Rating Scale (MADRS), Heart Rate Variability (HRV). Cortisol and adrenocorticotropic hormone (ACTH) were estimated at baseline and after ayurvedic therapy. HRV and endocrinal parameters were compared with age and gender matched healthy volunteers.

Results: HRV parameters showed significant sympathetic dominance in patients compared to healthy volunteers. Two months of ayurvedic treatment significantly decreased psychopathology, showed increase in vagal tone, decrease in sympathetic tone and reduced cortisol levels. However, there was no significant difference between groups receiving Ayushman A and B.

Conclusion: This study provides evidence for antidepressant, cardiac (HRV) and beneficial neuroendocrine modulatory influence of Ayurveda therapy in patients of Vishada (MDD). Further studies are needed to confirm these findings. Greater insight into the neurobiology behind this therapy might provide valuable information about newer drug target.

1. Introduction

Major depressive disorder (MDD) is the most common condition among patients seeking treatment with complementary and alternative therapies (CAM; Saeed et al., 2010). Despite effective allopathic antidepressant management plans, complete remission rate with allopathic pharmacotherapy remains low (Berton and Nestler, 2006; Thase, 2009). Ayurveda is among the oldest Indian indigenous systems of medicine with documented history of about 5000 years and 80% of the population still depends upon Ayurveda for their health concerns (Goyal et al., 2011). Although, evidence base for the efficacy of the majority of CAM interventions used to treat depression remains poor (Van der Watt et al., 2008), Ayurveda and its therapeutic strategies have promising potential especially in management of MDD (Conboy et al., 2009). But efficacy, safety and neurobiology of mechanism of

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http://dx.doi.org/10.1016/j.ajp.2014.07.002
1876-2018/

action of these treatments still remain to be evaluated due to lack of systematic research in this area (Qureshi and Al-Bedah, 2013; Ravindran and da Silva, 2013).

Major depressive disorder (MDD) is analogous to a disease condition described in Ayurveda as Vishada. According to Ayurveda, Vishada is a condition characterized by global reduction in activities of mind, sensory faculties and body (Dalhana, SS. Ka 3/21) arising out of disequilibria in doshas (CS su 9/4). It is defined in Ayurveda as a psychiatric disease (CS 25/40) Vishada has been mentioned as an extraordinary disease of the vata [nanatmaja vata vyadhi (CS su 20/11)]. The first chapter of Bhagawad Geeta, Arjuna vishada yoga probably records the first ever statement of symptoms of a patient suffering from Vishada [Veda Vyasa 1935]. Ayurveda literature has dealt with the disease and its management comprehensively.

There is a high association of cardiovascular dysfunction with depression. So also in äyurveda, soka (a synonym of Vishada) is mentioned as an important etiological factor of vätaja hradroga (heart disease) and Vishada as a symptom of vätaja hradroga.

Evidences have shown that lifetime history of depression predisposes a person to cardiovascular abnormalities (Bivanco-Lima et al., 2013). In patients of coronary heart disease with co morbid depressive disorder, the risk of subsequent mortality is higher (Musselman et al., 1998). One of the most plausible explanations for the increased cardiovascular mortality and morbidity in depression is the dysfunction of the central cardiac autonomic control particularly in vagus (Udupa et al., 2007a). Interestingly, heart rate variability (HRV) has been established as a sensitive measure of central cardiac autonomic control. HRV is being extensively used to investigate central regulation of autonomic state and to study the link between psychological process and physiological functions (Thayer et al., 2012).

Several investigators have demonstrated neuroendocrinal aberration in patients with MDD (Brown, 1989; Tichomirowa et al., 2005). Studies have demonstrated that certain subsets of patients are genetically predisposed for dyregulation in HPA axis (Brown, 1989; Herbert, 2013; Pariante and Lightman, 2008). Recent study conducted in healthy volunteer has shown that *Bacopa monnieri* an ayurvedic medication possess to reduce cortisol level (Benson et al., 2013; Tichomirowa et al., 2005). Similarly, several molecules present in ayurvedic medication might have antidepressant, sedative, and adaptogenic actions. However, literature on this issue remains scant.

In this study we evaluated of influence of ayurvedic treatment on psychopathology, HRV and endocrinal parameters in patients with major depression.

2. Methods

The study was a prospective, randomized, single blind, two group pre–post-test design. Drug naive patients diagnosed as Vishada by a qualified ayurvedic physician (comparative symptomatology description between Ayurveda and DSM is provided as supplementary material 1) and major depressive disorder according to DSM IV-TR by experienced psychiatrists, by thorough clinical interview, physical examination and relevant laboratory investigations were recruited from the outpatient department of National Institute of Mental Health and Neuro Sciences, Bangalore, India. Patients fulfilling the ayurvedic criteria for diagnosis of Vishada [Madhyama lakshana avastha: Charaka Samhita Vimana Sthana, 8th Chapter, 123rd Shloka, chakrapani tika. pp: 280–281], scoring >17 in Hamilton depression rating scale (HDRS) were randomly assigned into two groups. One group received Ayushman 15A and the other group received Ayushman 15B (using computer generated random numbers).

Patients not on any psychotropic/cardiotropic drugs for at least four weeks prior to entering the study were included. Patients with co-morbid psychotic disorder, suffering from any other known cardiorespiratory, neurological or endocrinological disorders as assessed by history and detailed clinical examination, co-morbid anxiety disorder, already on some anti-depressive therapy, comorbid substance abuse, pregnant/lactating mother were excluded from the study.

One hundred and four patients of MDD were recruited for the study out of which 23 patients dropped out and 81 patients completed the study (details are provided in Fig. 1). The age and gender matched normal controls were selected based on clinical interview and physical examination, from staff of NIMHANS who volunteered for the study. In patients symptom severity was assessed using Hamilton Depression Rating Scale (HDRS), Montgomery Asberg Depression Rating Scale (MADRS), CGI (clinical global impression). Study was approved by Institutional Ethics Committee. Written informed consent was obtained from all the participants.

Patients were administered following Ayurveda treatment module called as Virechana (therapeutic purgation). The module consisted of three steps (Purvakarma, Pradhanakarma and Paschatkarma).

2.1. Purvakarma (preparative procedure)

Pürvakarma consists of two therapeutic modalities, snehana (oleation therapy) and svedana (fomentation therapy). Firstly, the patient is administered sadyo äbhyantara snehana with Kshira peya ghrutadya ushna (sugared milk and ghee) (A Hr. Su 16/40-43) wherein, the patient is advised to consume 100 ml of milk with 10 g of dissolved sugar at 7 am in empty stomach followed by bähya snehana (self oil massage with sesame oil) and secondly, bhaspa sveda (steam) where the patient, is made to sweat moderately for 10 min. Rest of the day patient is on prescribed diet.

2.2. Pradhana karma (main procedure)

Pradhana karma consists of sodhana (therapeutic cleansing) and in this context virecana (therapeutic purgation). On the day two after undergoing bähya snehana and bhaspa sveda the patient is given a tablet of abhayädi modaka (Sha. S. uk. 4/27-34 procured from Zandu Pharmaceuticals) 500 mg in empty stomach at 8 am in the morning. This induces purgation for 4–8 times, during which patient is only on oral rehydration fluids.

2.3. Paschat karma (post-cleansing procedure)

The number of Virechana vegas (purgative episodes) induced by abhayadi modak 500 mg was 4–5 times which falls under avara vega (mild therapeutic purgation). Therefore ekannakala samsarjana karma (one meal time regimen) was administered. The pascat karma consists of post virecana dietary regimen for five meal times starting from the evening meal of day two to morning meal time of day three as follows: 1st annakala (meal time): peya (boiled rice water); 2nd annakala: vilepi (little boiled rice mixed with water), 3rd annakala: yusha (soup made with green gram), 4th annakala: rasa (mamsa rasa for non-vegetarians and pulses soup for vegetarians) and samanya bhojana was given in the 5th annakala (CS Si. 1/11, Chakrapani tika. pp: 678–679). This modulation in the Virechana karma was followed based on charaka's reference that the physician may as adopt modulated therapies (vikalpa) based on the circumstance giving due importance other parameters of assessment (CS Vi 8/125-126 chakrapani).

There was a limitation with respect to patient compliance with classical Virechana which would take at least 13–15 days. As the

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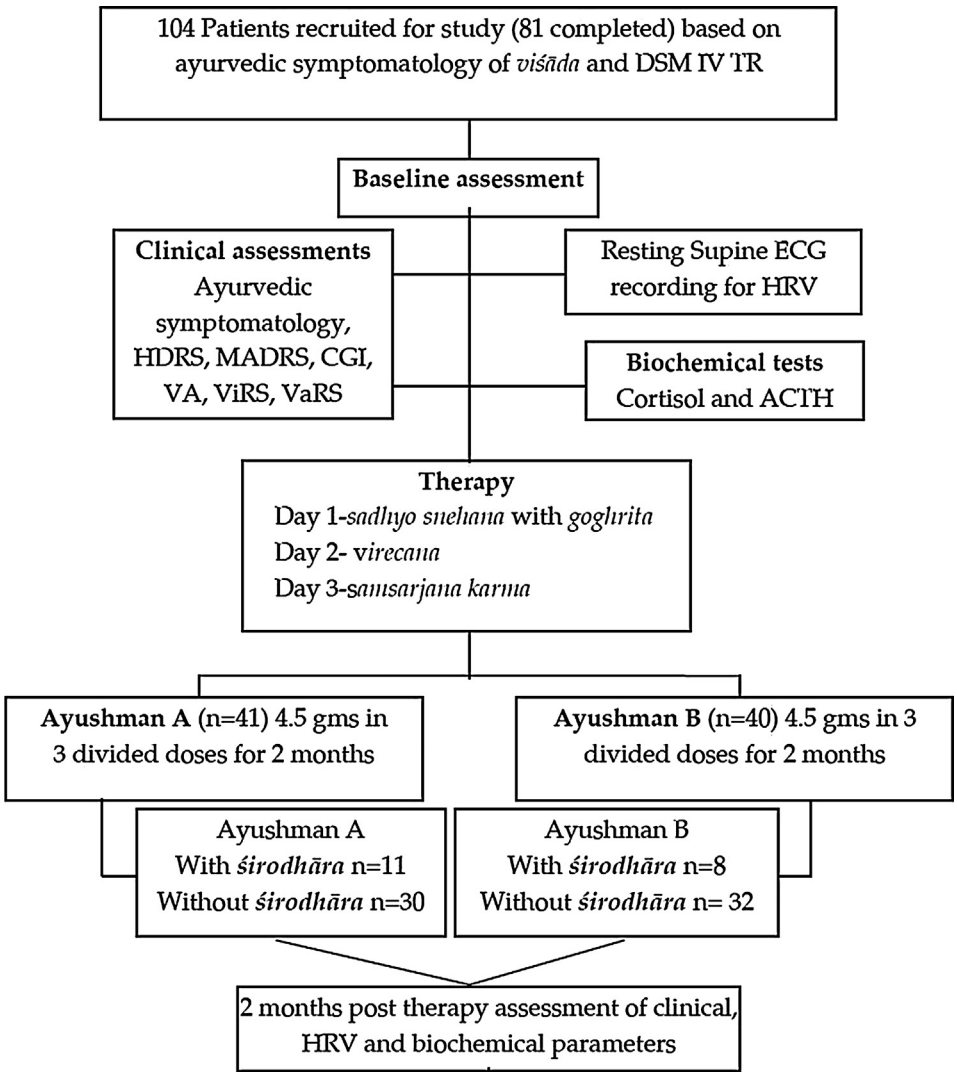

Fig. 1. Flowchart on details of study procedure and assessments.

trial was on patients of vishada it would naturally mean they would be of alpa satva and classical Virechana was an issue of compliance. Hence sadyo sneha and sukha virechana was used.

2.4. Oral medication

From day four, patients who had undergone sodhana were randomly divided into two groups, group A ($n = 41$) received Ayushman-15A (with Ashwagandha) 4.5 g per day (1.5 g three times a day) for two months and group B ($n = 40$) received Ayushman-15B (without Ashwagandha) 4.5 g per day (1.5 g three times a day) for two months. The formulations Ayushman-15A and Ayushman-15B comprising of medhya rasäyanä were supplied to us by the Central Council for Research in Ayurvedic Sciences (CCRAS) Govt. of India. Ayushman-15A contained an add-on drug ashvagandha. Sterile, 1.5 g unlabelled packets of formulations were dispensed to the patients and they were advised to consume the contents of 1 packet, half an hour before meal time three times daily (breakfast, lunch and dinner), with milk or honey or hot water.

2.5. Shirodhara

Along with the oral medication, 11 patients of group A received Sirodhära and 8 from group B received Shirodhara (Vaidya Haridas Kasture, 1999) for 7 days with Kshirabala taila (a ayurvedic medicinal oil preparation single avartita potency).

2.6. Patient monitoring

Reliable patient attenders (first degree relatives) were advised to monitor if the patients were taking the medicines as prescribed. During the period of Virecana they were advised to call the research fellow on telephone daily for three days and report about the developments regarding frequency of bowel movements and general health. During the course of taking oral medicines they were advised to call and report if there was any problem with the compliance or if the patient developed any new symptom and case of females if they became pregnant. Patients were given the packets sufficient for two months and asked to come for general assessment after one month and detailed assessment after two months.

2.7. HRV measurement

HRV measurement was carried out in Autonomic laboratory, Department of Neurophysiology, NIMHANS under standardized conditions. Subjects were instructed to abstain from caffeine and nicotine for at least 24 h prior to the cardiac autonomic function tests. Recordings were done after fifteen minutes of supine rest. In

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female patients, recording was done in proliferative phase of menstrual cycle. Lead II ECG and breathing signals were conveyed through analog digital converter (Power lab, 16 channels data acquisition system, AD Instruments, Australia) with a sampling rate of 1024 Hz. MLS 310 Module was used to analyze the different HRV measures. HRV was recorded and analyzed as per the guidelines of Taskforce report (1996). The data were analyzed offline using an automatic programme that allowed visual checking of the raw ECG and breathing signals. It was ensured that patients were breathing at normal respiratory rate of 12–15 breaths/min by recording the respiratory movements. Fifteen minutes basal recordings were stored and later analyzed to obtain time domain and frequency domain parameters of HRV. Further details of data acquisition are giving elsewhere (Abhishekh et al., 2013a,b; Udupa et al., 2007b).

2.8. Biochemical analysis

Serum cortisol and ACTH levels were estimated before and after antidepressant therapy. The samples were taken with all sterile precautions. After centrifugation the serum was separated and Aprotinin was added and then stored at −80 °C till the analysis. The samples were analyzed for serum cortisol and ACTH levels by enzyme amplified immuno-chemiluminescence method using kits from The Diagnostics Production Co., USA. Blood specimens were collected 2 h after light breakfast, at 8–9 am, after 30 min of supine rest in the lab. Samples were stabilized with EDTA (1 mg/ml blood) and aprotinin (300 KIU/ml blood) and cold (4 °C) centrifuged at 4000 rps for 15 min. After aliquoting, plasma was stored at −80 °C before being assayed for neuroendocrine variables. Plasma cortisol and ACTH levels were estimated using enzyme-amplified chemiluminescence method (DPC, USA).

2.9. Statistical analysis

Parametric tests were applied to the normal data, log transformed normal data and non-parametric tests were applied to the non-normal data. Repeated measures analysis of variance (RMANOVA) and post hoc least significant difference (LSD) tests were used to assess the within group (occasion effect – $p1$) and between group (interaction effect – $p2$) effects of the normal data. Wilcoxon signed ranks test was used to test the within group effects of the two major groups (groups A and B) as well as of the four subgroups (groups A0, A1, B0 and B1). Mann–Whitney tests were used to test the between group effects of groups A and B and Kruskal–Wallis test was used to test the between group effects of groups A0, A1, B0 and B1. A $p < 0.05$ was considered significant in all the tests. Pearson's correlation coefficient and was used to analyze the correlations between the HRV and clinical parameters. Hedge's Bias corrected effect size was computed to find the add-on effect of asvagandha on study parameters. Chi-square/Fisher exact test has been used to find the significance based on number of patients satisfying defined criteria for the study parameters. Odds ratio has been worked out to find the add-on effect of asvagandha on study parameters. Multivariate regression analysis has been used to evaluate the clinical parameters for measuring the effect of asvagandha some on parameters, which are selected, based on performance of these parameters using univariate analysis.

3. Results

Out of 104 subjects recruited into study, 81 subjects completed the study. There were 23 dropouts. The reason of drop out has been due to random events such as relocation or family circumstances (details given in supplementary material 2). Among 81 subjects, 41 who belonged to group A received Ayushman-15A (with asvagandha) and 40 who belonged to group B received Ayushman-15B (without asvagandha). Group A was again subdivided into group A0 who did not receive Shirodhara ($n = 30$) and into group A1 ($n = 11$) who received Shirodhara. Similarly group B was again subdivided into group B0 ($n = 32$) who did not receive Shirodhara and group B1 who received Shirodhara ($n = 8$).

Table 1
Subject characteristics of study group and healthy controls.

Variable	Depression group ($n = 81$)	Healthy controls ($n = 40$)	t/chi-square value	p value
Age in years	31.88 ± 8.55	30.73 ± 7.11	0.735	0.464
Gender (M:F)	46:35	26:14	0.749	0.253
Weight (kg)	60.22 ± 11.54	61.48 ± 9.95	−0.590	0.556
Body mass index	23.45 ± 4.18	22.79 ± 3.3	0.874	0.384

Patients in the study group and the drop-out group were statistically comparable with respect to age, gender, education, occupation, socioeconomic status, duration of illness, body mass index, clinical parameters and basal cardiovascular parameter (Supplementary Table 1). Patients in study and drop-out group were statistically comparable with respect to time and frequency domains of HRV parameters (Supplementary Table 2).

81 patients of depression were compared with, 40 age and gender matched normal controls. Both groups were identical in age, gender, and weight and body mass index (Table 1). Patients of depression group had significantly lower values of NN50 (number of RR intervals less than 50 ms) when compared to normal controls. The values of RMSSD (square root of the mean squared differences of successive intervals) and PNN50 (percentage of NN50) were low in patients of depression nearing significance when compared to normal controls indicating a low parasympathetic activity. The above said parameters had small effect size values while mean NN, NN50 and pNN50 had negligible effect size. Patients of depression group showed a relatively high sympathetic activity and a low parasympathetic activity as evidenced by significantly higher values of, LFnu (low frequency power in normalized units), SVB (sympathovagal balance or low frequency to high frequency ratio) and significantly lesser values of HF (high frequency power), HFnu (high frequency power in normalized units) respectively, when compared to age and gender matched normal controls. The parameters LFnu, HFnu and LF/HF ratio had a large effect size; HF power had small effect size. TP (total power) and LF power has negligible effect size (Table 2).

Depression group patients were randomized into two groups; group A ($n = 41$) and group B ($n = 40$). Group A received Ayushman A (with ashvagandha) and group B received Ayushman B (without ashvagandha) for two months. The parameters were assessed before and after the study. The results presented are to demonstrate the add-on effect of ashvagandha on the different parameters of the study. Patients of depression in both the groups were statistically comparable in terms of age, gender, height, and weight and body mass index. Duration of illness and family history were statistically comparable between two groups (Supplementary Table 3).

There was a significant decrease in the HDRS ($p < 0.001$), MADRS ($p < 0.001$) and CGS ($p < 0.001$) after therapy. The reduction in the mean scores of CGS in group A (from 4.05 ± 0.59 before treatment to 2.17 ± 0.74 after treatment) was significantly better ($p = 0.036$) when compared to group B (4.08 ± 0.69 before treatment to 2.55 ± 0.88 after treatment) (Table 3).

There was a significant increase in the mean values of mean NN ($p1 = 0.008$) and RMSSD ($p1 < 0.001$) after the therapy in both the groups. The NN50 values increased significantly following the therapy in both the groups ($p1 < 0.001$, $p2 = 0.030$) individually

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Table 2
shows HRV parameters in depression group and healthy controls.

Time domain parameters	Depression ($n=81$)	Healthy group ($n=40$)	p value	Effect size
Mean NN (ms)	819.63 ± 13.79 (556.6–1198)	801.00 ± 14.57 (606.9–961.9)	0.402	0.16 (N)
SDNN (ms)	44.68 ± 2.50 (10.1–109.18)	44.54 ± 2.46 (6.39–86.04)	0.972[+]	0.01 (N)
RMSSD (ms)	30.42 ± 2.14 (3.16–115.25)	36.96 ± 2.64 (3.74–82.22)	0.070[+]	0.35 (S)
NN50[a] (count)	37.50 ± 4.97 (0–215)	57.33 ± 8.17 (0–223)	0.032[*]	0.42 (S)
pNN50[a] (%e)	12.60 ± 1.58 (0–61.01)	18.31 ± 2.80 (0–65.7)	0.058[+]	0.37 (S)
Total power (TP) (ms^2)[b]	2132.37 ± 237.25 (84.5–10383.4)	2116.14 ± 244.06 (58.3–7887.8)	0.558	0.01 (N)
Low frequency power (LF) (ms^2)[b]	659.58 ± 72.15 (13.02–2840.84)	599.15 ± 87.63 (29.09–2450.33)	0.868	0.10 (N)
High frequency power (HF) (ms^2)[b]	461.80 ± 73.57 (2.55–4871.83)	626.21 ± 120.00 (14.87–4620.74)	0.046[*]	0.23 (S)
Low frequency power normalized units (LFnu)	58.32 ± 1.45 (31–82.20)	45.71 ± 2.10 (8.95–66.55)	<0.001[***]	0.95 (L)
High frequency power normalized units (HFnu)	33.97 ± 1.50 (8.41–64.06)	44.94 ± 1.95 (25.95–68.70)	<0.001[***]	0.83 (L)
Sympatho vagal balance (LF/HF ratio)	2.24 ± 0.17 (0.51–7.09)	1.15 ± 0.09 (0.33–2.44)	<0.001[***]	0.83 (L)

Results are presented in mean ± SE (min–max); NC: normal controls; N: negligible effect, S: small effect, M: moderate effect; L: large effect; VL: very large effect.
[a] Non-parametric test Mann–Whitney U test.
[b] Square root transformed.
[+] Nearing significance.
[*] Significant.
[***] Highly significant.

and in the sample as a whole before and after therapy ($p3 < 0.001$). The same was true with pNN50 in case of individual groups ($p1 = 0.009$, $p2 = 0.039$) as well as in the sample as a whole before and after ($p3 = 0.001$) therapy. There was a significant increase in the mean values of HF ($p1 = 0.001$) and HFnu ($p1 < 0.001$) after the therapy in both the groups but there was no significant difference between the groups. LFnu decreased significantly in both the groups individually ($p1 < 0.001$, $p2 = 0.007$) and the sample as a whole ($p3 = 0.001$) but there was no significant difference between the groups. LF/HF ratio decreased significantly in both the groups but there was but there was no significant difference between the groups. The outcome of add on therapy with ashvagandha was significantly high in parameters of SDNN, total power and CGS when compared to the other group without ashvagandha. These parameters also had moderate effect size. This indicates enhanced effect of ashvagandhä in increasing the parasympathetic activity (Table 4).

The percentage increase in values of SDNN [odds ratio (OR) = 2.38; $p = 0.058$], total power [odds ratio (OR) = 3.01; $p = 0.018$] and percentage decrease in values of low frequency power normalized units [odds ratio (OR) = 3.08; $p = 0.049$*] in responders of group A were significantly higher than in group B. This indicates enhanced effect of ashvagandha in increasing the parasympathetic activity and in decreasing the sympathetic activity.

Plasma cortisol levels of drug naive patients of depression at baseline ($n = 19$) [15.28 ± 5.25] were significantly high ($p < 0.001$) when compared to those of normal controls ($n = 20$) [6.94 ± 2.56]. There was no difference in the adrenocorticotropic hormone (ACTH) levels. The cortisol levels decreased significantly following two months therapy with Ayushman A ($p = 0.021$) (Table 5). The cortisol values of patients of depression after the therapy declined significantly but they were still significantly higher than the values of normal controls [controls: 6.99 ± 2.6 vs patients 12.85 ± 3.59; $p < 0.001$]. The ACTH values after therapy were significantly lower than those of normal controls [controls: 24.77 ± 7.18 vs 19.93 ± 6.44; $p < 0.05$].

4. Discussion

This study demonstrates positive influence of ayurvedic therapy on psychopathology, cardiac autonomic function and endocrinal parameters in patients with depression. So far the mechanisms of action of ayurvedic treatment has remained elusive because of dearth in studies and published reports. It remains a difficult to decipher the exact mechanism of action of ayurvedic formulations because rarely is a single herb extract is used in äyurveda. It is always a formulation consisting of multitude of crude herbs and other products. Identification of

Table 3
showing changes in clinical parameters before and after ayurvedic therapy.

Parameter	Ayushman A ($n=41$)		Ayushman B ($n=40$)		$F1$ $F2$ $F3$	$p1$ $p2$ $p3$
	Pre	Post	Pre	Post		
HDRS[*] (scores)	21.78 ± 0.59	11.05 ± 0.83	21.93 ± 0.51	10.8 ± 0.80	406.349 0.004 0.132	<0.001 0.049 0.718
MADRS[*] (scores)	27.49 ± 0.50	14.83 ± 0.89	27.6 ± 0.60	14.43 ± 0.89	596.684 0.026 0.238	<0.001 0.872 0.627
VAS[**] (scores)	8 (6–10)	4 (2–10)	8 (6–10)	4 (1–8)	−5.583 −5.484 −7.791	<0.001 <0.001 <0.001
CGS[**] (scores)	4 (3–5)	2 (1–5)	4 (3–5)	2 (1–5)	−5.612 −5.366 −7.732	<0.001 <0.001 <0.001

[*] Results presented as mean ± SEM; HDRS: Hamilton Depression Rating Scale; MADRS: Montgomery Asberg Depression Rating Scale; $F1$, $p1$: occasion effect (within groups), $F2$, $p2$: group effect (between groups); $F3$, $p3$: interaction effect (delta change between groups).
[**] Wilcoxon signed ranks test was used for within group effects. Results presented as median (max–min), VAS: Visual Analogue Scale, CGS: Clinical Global Impression-Severity. $Z1$, $Z2$ and $p1$, $p2$ are group wise break up values of group A and group B, respectively. $Z3$ and $p3$ are whole sample analysis values of within group effects.

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Table 4
shows modulation of HRV parameters after ayurvedic therapy.

Parameter	Group A ($n=41$)		Group B ($n=40$)		$F1$ $F2$ $F3$	$p1$ $p2$ $p3$	Effect size
	Pre	Post	Pre	Post			
Avg HR[a] (beats per min)	75.72 ± 1.80	73.37 ± 1.82	75.21 ± 1.83	71.61 ± 1.74	6.722 0.251 0.344	0.011 0.618 0.559	0.12 (N)
NN 50[b] (count)	25 (0–180)	60 (0–234)	20.5 (0-215)	49 (0-205)	−3.399 −2.169 −3.862	0.001 0.030 <0.001	0.32 (S)
pNN50[b] (%)	8.94 (0–56.6)	16.60 (0–75.24)	7.98 (0–74.91)	16.58 (0–66.08)	−2.624 −2.059 −3.288	0.009 0.039 0.001	0.22 (S)
Mean NN (ms)[a]	811.62 ± 20.47	836.36 ± 19.24	813.25 ± 18.82	857.08 ± 20.53	7.352 0.211 0.454	0.008 0.647 0.502	0.16 (N)
SDNN (ms)[a]	42.34 ± 3.09	50.33 ± 3.23	45.87 ± 3.74	44.39 ± 3.32	3.911 0.277 3.302	0.051 0.604 0.073	0.50 (M)
RMSSD (ms)[a]	30.77 ± 2.70	42.91 ± 3.86	33.36 ± 4.07	36.76 ± 3.67	15.341 0.566 1.380	<0.001 0.454 0.244	0.45 (S–M)
TP (ms^2)[a]	1952.14 ± 293.98	2678.47 ± 419.48	2308.86 ± 370.65	2097.99 ± 296.02	2.972 0.610 2.270	0.089 0.437 0.136	0.45 (S–M)
HF (ms^2)[a]	499.78 ± 95.88	914.41 ± 192.2	607.39 ± 156.51	694.37 ± 128.73	114.943 0.518 0.047	<0.001 0.474 0.829	0.36 (S)
LF (ms^2)[a]	631.19 ± 94.04	608.09 ± 76.05	579.41 ± 99.65	526.86 ± 103.04	0.013 1.149 0.399	0.908 0.287 0.530	0.05 (N)
LFnu (units)	56.54 (25.22–85.2)	40.28 (14.55–85.26)	55.18 (11.46–80.55)	41.89 (9.78–82.84)	−5.293 −2.702 −5.544	<0.001 0.007 <0.001	0.30 (S)
HFnu (units)[a]	37.17 ± 2.44	49.29 ± 2.66	37.33 ± 2.66	47.71 ± 2.66	37.217 0.013 0.222	<0.001 0.909 0.639	0.10 (N)
SVB (LF/HF ratio)[c]	2.10 ± 0.26	1.24 ± 0.23	1.98 ± 0.23	1.39 ± 0.21	25.901 0.016 0.813	<0.001 0.899 0.370	0.17 (N)

[a] Log transformed; results presented as mean ± SEM; Avg HR: average heart rate; $F1$, $p1$: occasion effect (within groups), $F2$, $p2$: group effect (between groups); $F3$, $p3$: interaction effect (delta change between groups).
[b] Results presented as median (max–min); NN50: count of number of pairs of NN (normal to normal RR) intervals differing by >50 ms; pNN50: NN50 divided by total number of all NN intervals $Z1$, $Z2$ and $p1$, $p2$ are group wise break up values of group A and group B respectively.
[c] Square root transformed; HFnu: high frequency normalized units; LF: low frequency power; HF: high frequency power; TP: total power; $F1$, $p1$: occasion effect (within groups), $F2$, $p2$: group effect (between groups); $F3$, $p3$: interaction effect (delta change between groups).

active ingredient of each herb and what happens when they are combined is still a complex question. Nevertheless there have been few studies looking into this matter as follows. Most of the ingredients uses in Ayurveda are medhya rasayana (drugs that are useful in enhancing cognitive faculties). And contemporary studies on them have suggested that they have free radical scavenging, antistress and adaptogenic properties. Some examples are as given below.

Table 5
Effect of ayurvedic therapy on endocrinal parameters.

Variables	Group A ($n=9$)		Group B ($n=8$)		$F1$ $F2$	$p1$ $p2$
	Pre	Post	Pre	Post		
Cortisol (μg/dL)	16.54 ± 4.35	12.20 ± 1.89	14.56 ± 6.49	13.59 ± 4.91	6.623 0.022 2.657	0.021* 0.884 0.124
ACTH (pg/mL)	21.58 ± 4.68	25.35 ± 9.05	22.63 ± 4.09	24.19 ± 5.28	2.007 0.001 0.345	0.178 0.982 0.566

Results are presented as mean ± SEM; ACTH: adrenocorticotropic hormone; $F1$, $p1$: occasion effect (within groups), $F2$, $p2$: group effect (between groups); $F3$, $p3$: interaction effect (delta change between groups).

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Bacopa monniera and *Convolvulus pluricaulis* along with other herbs have been suggested as useful medhya rasäyanä and hold a great potential in the treatment of central nervous system disorders (Kumar, 2006). *Oscimum sanctum* has been evaluated for nootropic potential (Joshi and Parle, 2006). *Withania somnifera* has been the studied for wide variety of effects ranging from antioxidant, anti-stress, anti-inflammatory, anti-aging to adaptogenic, immunomodulatory, cognition-facilitating (Bhattacharya et al., 1997, 2000a,b,c, 2001; Bhattacharya and Ghosal, 1998). Cytoprotective role of *N. jatamansi* in oxidative injuries have been reported (Subashini et al., 2006) and protective effect of *Nardostachys jatamansi* on neurobehaviour and antioxidant properties have also been reported (Salim et al., 2003). Protective effect of *Convolvulus pluricaulis* has been reported (Panda and Kar, 2001). Likewise antistress and antioxidant action of *Bacopa monnieri* has also been reported. Further, there has been evidence of oxidative damage in the pathogenesis of depression (van Amsterdam and Opperhuizen, 1999) and neurodegenerative pathology as a result of oxidative stress (Avshalumov and Rice, 2002; Bremner et al., 2000). It has also been reported that antidepressant therapies like selective serotonin reuptake inhibitors have antioxidant action (Finkel et al., 1996). Therefore by implication, it is clear that antioxidant activity consistent with rasäyanä property prevents neurodegeneration and thus might act in correcting the pathophysiology in depression.

Although, in our study the clinical improvement in both the groups were comparable, the add on effect of asvagandha was evident by an enhanced response to Ayushman A than to Ayushman B in selected HRV parameters, viz., SDNN, total power, LFnu and in biochemical parameter cortisol. Significant increase in SDNN and total power indicates an add-on increase in parasympathetic activity. Similarly significant decrease in LFnu and in cortisol levels indicate an add on decrease in the sympathetic activity in group A. Ashvagandha might be acting centrally by altering the autonomic balance towards increased parasympathetic activity and peripherally by decreasing the cortisol levels whereas the formulation without ashvagandha has a more central effect than peripheral.

Although there was no significant difference between the groups, from baseline to endpoint both groups showed improvement in domains of psychopathology as well as biomarkers. Data provides evidence for beneficial neurobiological effects of the treatment. However, we do not have data regarding the exact molecule responsible for this clinical phenomenon. Nevertheless, ayurvedic treatment will contain several molecules which may have differential agonist and antagonist activity on the receptor. It may be difficult to characterize such interaction. Further animal studies are needed on this aspect.

Certain limitations and caveats should be kept in mind while interpreting results. Inclusion of a patient group comprising of established antidepressant therapies or a placebo control would have made the study more robust. Hormonal analysis could be performed on a small group of patients due to logistic difficulties. Also, interpretation of single plasma cortisol levels may not be accurate and the current standard is to do serial measurements for several hours. However it was just sufficient to add a perspective to the study. We do not have data on long-term side effect of this therapy. Further studies are needed particularly to assess nephrotoxicity and hepatotoxicity.

In conclusion, this study provides evidence for antidepressant, cardiac and neuroendocrine modulatory influence of Ayurveda therapy. Further studies in larger sample are needed to confirm these finding. Greater insight into the neurobiology behind this therapy might provide valuable information about newer drug target. Phytochemical and Pharmacological studies on these ingredients individually and then in combination could help decode the exact mechanism of action.

Acknowledgements

The authors are grateful to Central Council for Research in Ayurvedic Sciences, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India, for funding the study and National Ayurveda Dietetics Research Institute (Formerly Regional Research Institute under CCRAS) Bangalore for supplying the AYUSHMAN - 15 formulations.

Appendix A. Ayurvedic references

Abbreviations of ayurvedic references

CS: Charaka Samhita; SS: Sushruta Samhita; Sha. S: Sharangadhara Samhita; AH: Ashtanga Hridaya; BG: Bhagavat Geeta; uk: Uttara Khanda; su: sutra sthana; chi: chikitsa sthana; si: siddhi sthana (*Note*: numbers following ayurvedic abbreviations have been given as *x*/*y*–z: where x is the name of the chapter, *y*–*z* are numbers of verses).

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ajp.2014.07.002.

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