

Comparative Clinical Verification Of Wild And Cultivated *R. Serpentina* Mother Tincture In Stage I Essential Hypertension

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Abstract

One of the most efficient homoeopathic treatment for hypertension is *R. serpentina* mother tincture due to presence of indole alkaloid *Reserpine*. It is overexploited and commonly harvested for commercial purposes which lead to its extinction. As a result it has now become an endangered plant species in India so manufactures are using cultivated species. Consequently there is a need to scientifically validate comparative clinical verification of wild and cultivated *R. serpentina* mother tincture in mild essential hypertension. This study was planned for comparative clinical verification of wild and cultivated *R. serpentina* mother tincture in stage I essential hypertension. Phase II, double blind, randomized, parallel assignment, monocentric, clinical trial was undertaken on the population suffering from Stage I essential hypertension. Out of 503 screened patients, 108 patients were voluntary recruited which fulfill the case definition, inclusion criteria, exclusion criteria. 108 patients suffering from stage I essential hypertension; randomized to receive either wild MT (n = 104) or cultivated MT (n = 104). The therapeutic efficacy of wild and cultivated *R. serpentina* mother tincture in patients with stage I essential hypertension has been statistically proven. However, effect observed in wild sample are higher than cultivated sample. *R. serpentina* MT from wild and cultivated species has significantly favorable effect in patients suffering from stage I essential hypertension but *R. serpentina* MT, from wild species was relatively more effective than those from cultivated ones. To overcome the issue of extinction of *Rauwolfia*, its cultivation is crucial and further researches must be done to enhance the therapeutic value of cultivated species.

Keywords: *R. serpentina*, Mother tincture, Hypertension, HRQoL, m- Minichal scale, Clinical trial

1. INTRODUCTION:

Essential hypertension (also known as primary hypertension or idiopathic hypertension) is the most common type of hypertension, affecting 95 percent of adults. It's more common in hypertensive patients with a family history, and it might happen as a result of environmental and hereditary factors^[1]. Globalization, sedentary lifestyles, obesity, unhealthy diets, dangerous alcohol use and occupational stress all contribute to increasing anxiety which ultimately produces mental and emotional stress. All of these elements play an important role in the development of HTN^[2]. Hypertension is one of the topmost causes of death worldwide. According to the World Health Organization, hypertension affects one out of every five persons. High blood pressure is a key risk factor for cardiovascular disease, accounting for approximately 54 percent of strokes and 47 percent of coronary heart disease over the world^[3]. As a result, we may assert that having high blood pressure is a major risk factor for cardiovascular disease. It is time to pay greater attention to the treatment of patients who already have high blood pressure. Even a minor reduction in the blood pressure would alleviate most of the existing burden of BP-related CVD. Compliance with antihypertensive treatment is very poor. Compliance defined as the extent to which a patient's behavior conforms to medical advice. Antihypertensive medication regimens have limited long-term compliance, according to Cohen JS 2001^[4]. Many antihypertensive drugs cause ADEs even at therapeutic doses, such as dizziness, headaches, constipation, low energy, sedation, and sexual dysfunction etc.^[5]. Accordingly, Doctors must have to consider Health-related quality of life (HRQoL) while administering antihypertensive medications and their dosage due to the high occurrence of ADEs. The expense of drugs and adverse drug events are two of the most common reasons for poor compliance among patients using antihypertensive medicines^[6-11]. In the case of hypertension, homoeopathy is highly recommended because it not only lowers and stabilizes blood pressure but also improves HRQoL. One of the most

efficient homeopathic treatment for hypertension is *R. serpentina* mother tincture due to presence of indole alkaloid *Reserpine* [12-14]. It is overexploited and commonly harvested for commercial purposes which lead to its extinction. As a result, it has now become an endangered plant species in India so manufactures are using cultivated species. Nevertheless, there is claim that wild species of medicinal plants are more potent than the cultivated plants [15-16]. Consequently, there is a need to scientifically validate comparative clinical verification of wild and cultivated *R. serpentina* mother tincture in stage I essential hypertension. Phase II, double blind, randomized, parallel assignment, monocentric, clinical trial was undertaken on the population suffering from stage I essential HTN as per the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guidelines 2018 [17].

2. MATERIALS AND METHODS:

2.1 Trial Design:

Phase II, double blind, randomized, parallel assignment, monocentric, clinical trial was undertaken on the population suffering from Stage I essential HTN as per the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guidelines 2018.

2.2 Ethical Consideration:

IEC approval was taken prior to the start of clinical trials. The trial was registered in the Clinical Trials Registry- India (CTRI Registration No: CTRI/2020/03/024231). Guidelines by ICMR (Indian Counsel of Medical Research), ICH (International Council for Harmonization) were followed in this study.

2.3 Inclusion and Exclusion Criteria:

Patients aged between 30 and 60 years, both genders, suffering from Stage I essential HTN (SBP 140–159 mmHg; DBP 90–99 mmHg) and willing to participate in the study were included. Patients not willing to participate, who had established secondary hypertension, or patients with damage to target organ, women who are taking contraceptive pills, pregnancy and lactating mother, who had history of hypersensitivity of *Reserpine* were excluded from trial.

2.4 Medicine:

R. serpentina mother tincture (wild and cultivated) were prepared at Medisynth Ch. Pvt. Ltd. as per guidelines of Homeopathic Pharmacopoeia of India. Medicines were stored in Bharati Vidyapeeth Homeopathic Pharmacy, Katraj, Pune - 411043. Mother tinctures were stored in amber colour glass bottle kept in dark place, under appropriate temperature, away from sunlight & humidity condition. 10 drops of the mother tincture administered orally with 1/4 cup of water for 7 days.

2.4 Sampling Procedure:

Double blind, lottery method.

2.5 Participants:

The patients of known case of mild essential hypertension were enrolled in this study. Out of 503 screened patients, 108 patients were voluntary recruited which fulfill the case definition, inclusion criteria, exclusion criteria.

2.6 Randomization:

Out of 503, 104 patients randomized in Group I which were received wild sample and 104 patients randomized in Group 2 which were received cultivated sample. From group I out of 104 patients 3 drop out from group II and out of 104 patients 3 drop out. Participants randomized by lottery method. The Pharmacist at Bharati Vidyapeeth Homeopathic Pharmacy, Katraj, Pune had assigned the patients to one of the intervention groups using lottery method of randomization technique. In this technique slips of papers of same size, shape and color are titled with wild MT or cultivated MT according to sample size. Then they were folded and mixed up in a container. Enrolled patients were requested for choose a slip from container. Accordingly, they were received wild or cultivated MT. The assigned groups were maintained throughout the study.

2.7 Measurement of blood pressure:

Measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP) was done using the routine method as per the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guidelines. Blood pressure (BP) was measured in sitting position by principal investigator. Before taking the BP, the patient was asked to sit quietly for at least 5 min in a chair with feet on the floor, rather than on the examining table. Three readings from both the arms were taken at an interval of 5 min. Then, the average of the three readings were calculated. The value obtained was considered for the inclusion of the patient into the study. Symptoms related to blood pressure including headache, insomnia, chest pain, palpitations and other were recorded using a questionnaire.

2.8 Primary outcome measure:

To evaluate the mean change in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from baseline and to evaluate the mean change in impact on health related quality of life (HRQoL) by m-Minichal score after intervention of wild and cultivated *R. serpentina* MT.

2.9 Secondary outcome measure:

To assess any adverse drug event (ADE) of homoeopathic medicine *R. serpentina* due to presence of Reserpine.

2.10 Auxiliary line of treatment:

Homoeopathic medicine with auxiliary line of treatment like life style modifications, salt restriction in diet, healthy diet, eight hours sleep, pranayam etc. were advised to enrolled patients. All participants were strongly encouraged to avoid or to stop alcohol and smoking if they did so.

3RESULTS:

3.1 Demographic Characters:

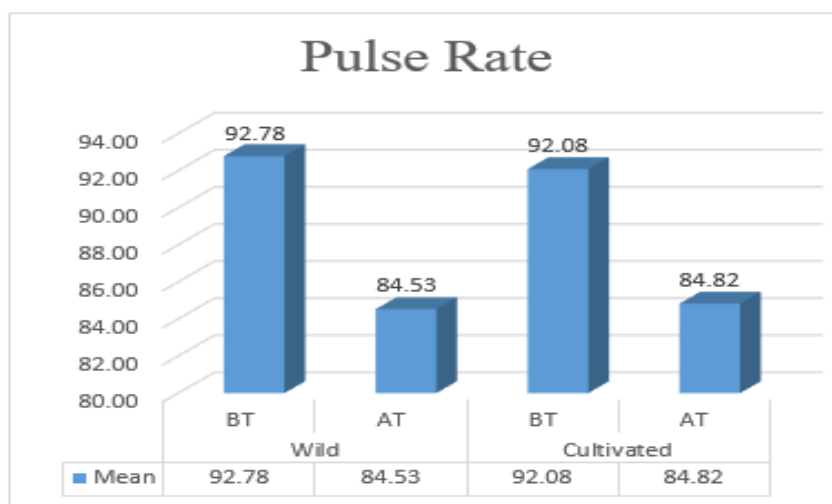
Various demographic parameters were evaluated in hypertensive patients. A total of 108 patients with hypertension were included in the study The highest number of patients were from age group of 51 to 60 years (40%) followed by the age group of 41-50 years (35%) and followed by 30-40 Years (25%). Gender wise distribution shows males and females were equally affected by hypertension (male 50% and Female 50%). The majority of patients were highly educated (education up to university level 76%, Secondary school 22% and uneducated 2%). According to occupation working people had, more incidence of hypertension may be due to stress, as stress is main causative factor in essential hypertension (service 55%, business 7 %, housewife 33% and retired 5%). Most of the patients (wild group 93.55 cultivated group 93.33) were living with their family (married 96%, unmarried 3% and widow 1%). In this study, most of obese and overweight patients were more affected as compared with patients with normal weight (obese 58%, over weight 33% and normal weight 9%). As in this study 41% of patients were with alcohol addiction, 29% of patient with smoking and alcohol addiction, 11% only smoking, 8% gutkha , 3 % misri, 11% with no history of any addictions.

3.2 Pulse Rate:

Pulse Rate		Mean	N	SD	SE	t-Value	P-Value	% Effect	Result
Wild	BT	92.78	51	5.46	0.76	6.976	0.000	8.90	Sig
	AT	84.53	51	4.64	0.65				
Cultivated	BT	92.08	51	5.04	0.71	9.729	0.000	7.88	Sig
	AT	84.82	51	5.19	0.73				

Table No. 1: Paired t-Test for pulse rate

For pulse rate, we used paired t-Test to test efficacy in both groups. P-Value for both the groups was less than 0.05. Hence effect observed in both groups were significant. Effect was 8.90% for the wild sample and 7.88% for the cultivated sample.



Graph No. 1: Paired t-Test for pulse rate

Pulse Rate	N	Mean Diff.	SD	SE	t-Value	P-Value	Result
Wild	51	8.25	4.98	0.89	2.037	0.046	Sig
Cultivated	51	7.25	3.57	0.65			

Table No. 1: Unpaired t-Test for pulse rate

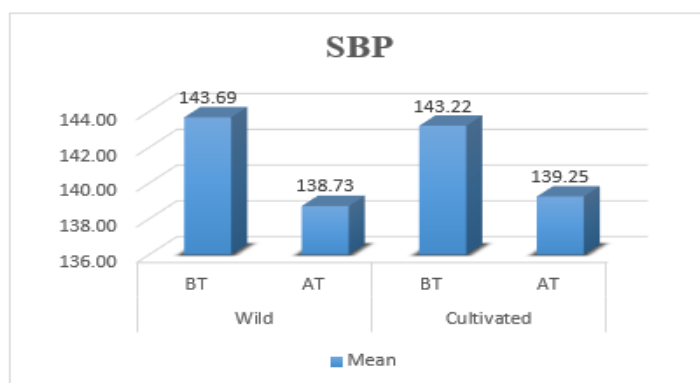
For comparison between two groups, unpaired t-test was carried out. P-Value was less than 0.05., hence there was significant difference between result observed wild sample and cultivated sample. The mean difference for wild sample was greater than cultivated sample.

3.3 Systolic Blood Pressure (SBP):

SBP		Mean	N	SD	SE	t-Value	P-Value	% Effect	Result
Wild	BT	143.69	51	2.38	0.33	8.618	0.000	3.45	Sig
	AT	138.73	51	2.26	0.32				
Cultivated	BT	143.22	51	2.00	0.28	9.520	0.000	2.77	Sig
	AT	139.25	51	2.04	0.29				

Table No. 2: Paired t-Test for SBP

For SBP, since observations are quantitative, paired t-Test was carried out to test efficacy in both groups. P-Value for both the groups was less than 0.05, hence effect observed in both groups was significant. Effect was 3.45% for the wild sample and 2.77% for the cultivated sample.



Graph No. 2: Paired t-Test for SBP

SBP	N	Mean Diff	SD	SE	t-Value	P-Value	Result
Wild	51	4.96	2.29	0.41	2.029	0.047	Sig
Cultivated	51	3.96	1.92	0.35			

Table No. 3: Unpaired t-Test for SBP

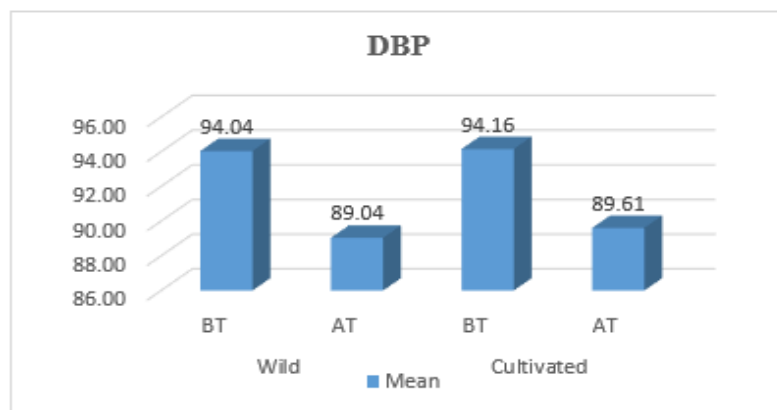
For comparison of SBP between two groups, unpaired t-test was carried out. P-Value was less than 0.05., hence there was significant difference between results observed in wild sample and cultivated sample. The mean difference for wild sample was greater than cultivated sample thus more of an effect was seen in the wild sample than in the cultivated sample.

3.4 Diastolic Blood Pressure (DBP):

DBP		Mean	N	SD	SE	t-Value	P-Value	% Effect	Result
Wild	BT	94.04	51	2.02	0.28	11.547	0.000	5.32	Sig
	AT	89.04	51	2.42	0.34				
Cultivated	BT	94.16	51	2.07	0.29	10.589	0.000	4.83	Sig
	AT	89.61	51	2.19	0.31				

Table No. 4: Paired t-Test for DBP

Since observations are quantitative, paired t-Test was used to test efficacy in both groups. P Value for both the groups was less than 0.05; hence, effect observed in both groups was significant. Effect observed in wild sample was 5.32%, and in cultivated sample was 4.83%.



Graph No. 3: Paired t-Test for DBP

DBP	N	Mean Diff	SD	SE	t-Value	P-Value	Result
Wild	51	5.00	1.65	0.30	2.152	0.035	Sig
Cultivated	51	4.55	2.34	0.43			

Table No. 5: Unpaired t-Test for DBP

For comparison of DBP between two groups, unpaired t-test was carried out. P-Value was less than 0.05, hence there was significant difference between result observed wild sample and cultivated sample. The mean difference for wild sample was greater than cultivated sample thus more of an effect was seen in the wild sample than in the cultivated sample.

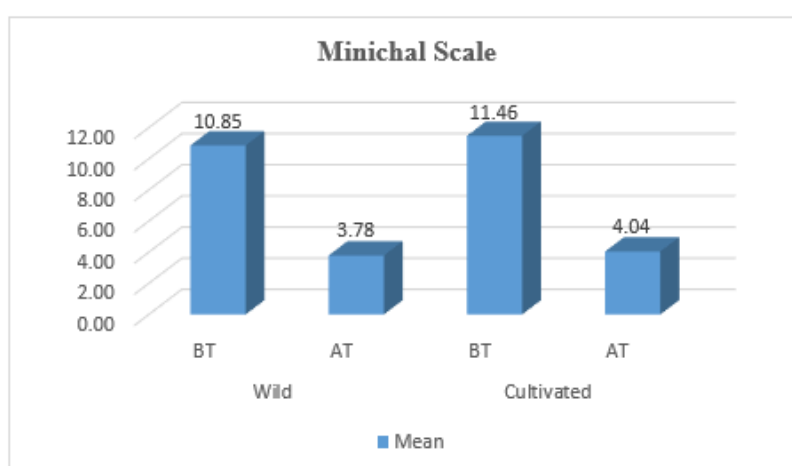
3.5 Health-related quality of life (HRQoL):

In this study HRQoL in patients with mild hypertension were assessed by m- Minichal scale. For m-Minichal scale reliability analysis were done independently on both group, chronbach's alpha value for wild and cultivated group was 0.766 and 0.737 respectively. So this tool was reliable as per chronbach's alpha value.

m-MINICHAL Scale	Mean	Median	SD	Wilcoxon W	P-Value	%Effect	Result	
Wild	BT	10.85	10.00	3.24	-3.873 ^a	0.00016	65.13	Sig
	AT	3.78	3.00	2.44				
Cultivated	BT	11.46	11.00	3.55	-3.806 ^a	0.00019	64.77	Sig
	AT	4.04	4.00	1.84				

TableNo. 6: Wilcoxon Signed Rank Test for m-MINICHAL Scale

Since observations are on ordinal scale (gradations), we have used Wilcoxon Signed Rank Test to test efficacy in both groups. From above table, we observed that, P-Value for both the groups is less than 0.05. Hence we concluded that, effect observed in both groups is significant. Effect observed for Wild sample is about 65.13% while 64.77% for cultivated sample.



Graph No. 4: Wilcoxon Signed Rank Test for m-MINICHAL Scale

Group	N	Mean Rank	Sum of Ranks	Mann-Whitney U	P-Value
Wild	51	52.61	2841.10	212.000	0.047
Cultivated	51	49.23	2658.60		
Total	102	101.84	5499.70		

Table No. 7: Mann Whitney U test for m-Minichal Scale

For comparison between two groups, Mann Whitney U test carried out. P-Value is less than 0.05, hence there was significant difference between results observed in wild sample and cultivated sample. Further we observed that, mean rank for wild sample is greater than cultivated sample. Thus, more of an effect was seen in the wild sample than in the cultivated sample.

4. DISCUSSION:

The extinction of plant species is a well-known consequence of relying primarily on wild resources for raw materials. Cultivation is therefore offered as a feasible alternative that offers an opportunity to get over the problems with medicinal herbs [18]. The 2017 study investigated and compared the *Rauvolfia* plant's in vitro antioxidant and anti-diabetic properties in both its wild and cultivated varieties. It is possible to conclude from the in-vitro research that a cultivated variety of the plant can be utilized in place of a wild variety of *R. serpentina* [19]. In the current study, *Rauvolfia serpentina* plant tissue were regenerated in vitro, and samples of the plant's roots, leaves, and callus were analyzed for alkaloids. The in vitro regenerated roots had the most reserpine, whereas the leaves contained the least [20]. In a previous study, researchers used LC-MS/MS analysis to determine the concentrations of reserpine in both wild and cultivated MT, which were found to be 0.548 mg/g dry wt. and 0.440 mg/g dry wt., respectively [21]. Accordingly clinical trial was conducted to examine comparative clinical verification of wild and cultivated *R. serpentina* mother tincture in stage I essential hypertension. In this study HRQoL in patients with mild hypertension were assessed by m- Minichal scale. For m-Minichal scale reliability analysis were done independently on both group, chronbach's alpha value for wild and cultivated group was 0.766 and 0.737 respectively. So this tool was reliable as per chronbach's alpha value. The therapeutic efficacy of wild and cultivated *R. serpentina* mother tincture in patients with mild essential hypertension has been statistically proven. In this trial authors found, wild and cultivated *R. serpentina* mother tincture not only lowers and stabilizes blood pressure but also improves HRQoL. The complaints like insomnia, headache, palpitation, pain in chest were reduced in most of the cases along with general improvement of the patient. No any adverse drug event was found during trial in both wild and cultivated MT. Significant improvement was seen in pulse rate, DBP, SBP and m-Minichal score with the P- value less than 0.05 for patients receiving wild and cultivated *R. serpentina* mother tincture, but effect observed for pulse rate, DBP, SBP and HRQoL in wild sample was relatively more than cultivated sample. To overcome the issue of extinction of *Rauvolfia*, its cultivation is crucial.

5. CONCLUSION:

R. serpentina MT from wild and cultivated species has significantly favorable effect in patients suffering from stage I essential hypertension but *R. serpentina* MT, from wild species is relatively more effective than those from cultivated ones. To overcome the issue of extinction of *Rauvolfia*, its cultivation is crucial and further researches must be done to enhance the therapeutic value of cultivated species.

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8. Conflict of interest statement:- Declaration of Competing Interest None declared.

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