RESEARCH ARTICLE

Standardization and Evaluation of Herbal Antihypertensive Sarpagandha (Reserpine) Tablet Formulations

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ABSTRACT

The need for herbal medicines is rising day by day tremendously all over the world. Many pharma industries are nowadays directing their studies on herbal material for their probable therapeutic values. In various scientific journals, a growing volume of research publications relied upon plant-based drugs and formulations. Hypertension is an important chronic disorder in economically developed as well as developing countries. According to WHO guidelines, it is necessary to standardize the herbal products that reach patients. Hence, standardizing the herbal antihypertensive formulations is necessary to give safe and effective medicines to the patients and society. The herbal antihypertensive formulation was standardized according to Ayurvedic Pharmacopeia and WHO guidelines. Various parameters such as botanical, physicochemical, pharmacological and toxicological were studied. A pharmacological study was carried to screen the selected marketed formulations for hemolytic and antihypertensive activity. The study showed that all formulations pass most of the standardization parameters and can be recommended for human beings. The outcome of present study will support the process of regulatory agencies and help in maintaining the quality of herbal medicines.

Keywords: Antihypertensive, Herbal formulations, Sarpagandha, Standardization.

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INTRODUCTION

In ancient times, herbal drugs were used as a medicine to treat a wide range of diseases. In recent decades, crude drugs from plant, animal or mineral origins are used for health care.[1] From last few years, there was an extraordinary requirement for plant-originated medicines in advanced nations.[2] Compared to synthetic products, natural products are widely used as medicines with the trust that they are harmLess with less or no side effects.[3] Those products, which are progressively utilized as cosmetics, nutraceuticals, and medicines, may have excellent synchronization within the quality of raw materials, in-process materials, and finished products. It takes time to design and produce sensitive, specific, and dependable quality control tactics using a combination of traditional and modern instrumental analysis methods.[2] According to the WHO, herbal and alternative therapies are used by approximately 70% of the world's population for healthcare.[4]

Standardization of herbal formulations is critical for assessing drug efficacy, quality, safety, and purity, which are all dependent on the concentration of active constituents.[4] The factors that contribute to the product's effectiveness, suitability, and security may have an impact on the overall quality of herbal drugs, either directly or indirectly. The identity of drugs is confirmed during standardization, and the purity and quality of drugs are estimated.[3] Standardization is regarded as a critical foundational tool in the quality control process.[2] For every

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plant medicine, the system of standardization is established because there is a possibility for enormous dissimilarity in various medicines.[3] Standardization techniques concentrate on all aspects that may contribute to the quality of natural drugs, such as accurate identification, phytochemical, biological, organoleptic, and toxicity evaluation.[2]

In ancient times, vaidyas were involved in treating the sufferers in an individual manner, and accordingly, treatments were given. Nowadays, generalized herbal medicines are available, which are associated with certain issues like shelf life, the high cost of production, purity and market distribution. These have demanded the improvement of cutting-edge and perfect standards for estimating the efficacy and quality of those drugs.[2]

Humans also are becoming privy to the strength and unexpected result. To retain civic trust and to convey a natural product into the flow of an available fitness care machine, the investigators, manufacturers, and regulatory authorities must practice rigorous medical practices to safeguard the great uniformity of conventional natural drugs.[2]

High blood pressure or high blood stress is not unusual and can cause or complicate many coronary heart issues. Hypertension (HTN) is frequently referred to as the "silent killer" as it generally has no serious signs and symptoms at the beginning but gradually becomes dangerous at the end. Hypertension is usually high blood strain (more than 140 mm hg) within the arteries. Hypertension is defined as a persistent increase in systemic arterial blood pressure.[5] In 2005, approximately 20.9% of women and 20.6% of men in India had hypertension, with rates expected to rise upto 23.6 and 22.9 for women and men, respectively, by 2025.[6]

Standardization parameters are divided into four categories: botanical, physicochemical, pharmacological, and toxicological.[7] Considering all of this, the current work was designed to standardise the herbal Sarpagandha (Reserpine) tablet formulation to achieve product quality. This work may be beneficial to strengthen the regulatory process.

MATERIALS AND METHODS

Chemicals and Instruments

Chloroform (99.8% AR), methanol (98.8% AR/ACS), acetone (99.5% AR/ACS) were all purchased from LOBA chemicals, Mumbai. Heparin, ketamine, normal saline was purchased from local medical shops. Marketed samples of Sarpagandha (reserpine) tablets of five different manufacturers were purchased from the local market and labeled as A, B, C, D, and E. Instruments used were a muffle furnace, a hot air oven, an electronic water bath, a bath sonicator, a hardness tester, a Vernier calliper, a sphygmomanometer, and a student physiograph/data acquisition system (the BIOPAC system) with pressure transducers.

Standardization of Antihypertensive Sarpagandha (reserpine) Tablet Formulations

The standardization of antihypertensive formulations was conducted as per standard guidelines/procedures. Several parameters like botanical, physicochemical, and toxicological parameters were determined as per standard procedures/guidelines except pharmacological parameters.[8,9]

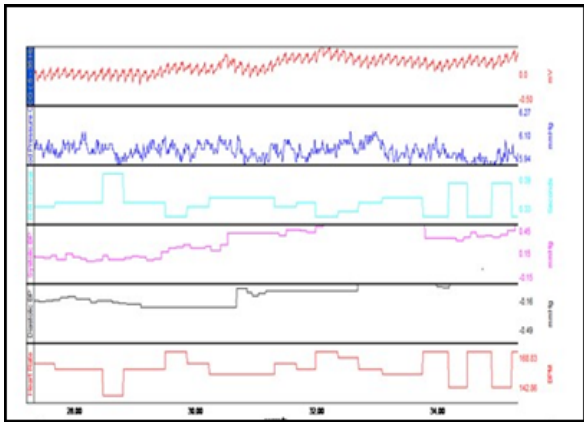

Figure 1: The baseline recording.

Pharmacological Parameters

Antihypertensive Study

Healthy adult wistar albino rats (180–200 gm) of male or female sex amid 2 to 3 months of oldness were utilized for the investigation. These rats were kept in a set of polypropylene enclosures, preserved under normal settings (12 hours light-dark series, temperature 25 ± 1°C, RH 40–60%), and nourished with pure water and food in the form of a rat pellet. Trials were completed at Bharati Vidyapeeth College of Pharmacy, Kolhapur, in accordance with the CPCSEA guidelines given by the Ministry of Environment and Forests and Climate Change, New Delhi. Protocol with proposal number BVCPK/CPSCEA/IAEC/01/05/2017-0218 was sanctioned from the Institutional Animal Ethics Committee (IAEC).[10]

Stock solutions of drug such as formulation A (1), B (1), C (1), D (1), E (1 mg/mL) and normal saline were prepared freshly for work and their effects on BP were compared. From each stock solution, the working standard of suitable concentration was prepared. Also, pyrogen-free distilled water was used for making drug solutions. Herbal formulations were tested for acute toxicity study per OECD guidelines 423 procedure. For this study, 15 male and female rats aged between 3 to 5 months and weighing 180 and 200 g were used. Five groups of animals were created: the first group (3 animals) was kept as a control, the second group (3 animals) was treated with standard, and the remaining three groups (a total of 3 animals per group) were used for the testing of the formulations.[11] After cannulation, the animal was kept in the student physiograph/data acquisition system (BIOPAC system) to record ECG and BP. The entire system was stabilized for 10–20 minutes, if there was bleeding it should be controlled. To ensure the stability of the preparations, baseline recording (Figure 1) was carried out for 10–15 minutes. After the administration of 0.1 mL of normal saline, the chosen dose of drug solutions 0.1 mL was administered via the tail vein/femoral vein to finish the drug administration. The effect and period of action of drug were detected, and BP of animal was permitted to return to its

Table 1: The result of macroscopy

Formulation	*Colour*	*Odour*	*Taste*	*Texture*
A	Greenish	Bitter/pungent	Bitter	Smooth
B	Pale greenish	Bitter/pungent	Bitter	Smooth
C	Pale brownish yellow	Bitter/pungent	Bitter	Smooth
D	Greenish	Bitter/pungent	Bitter	Smooth
E	Brownish	Bitter/pungent	Bitter	Smooth

Table 2: The result of weight variation, friability and hardness

Formulation	*Weight variation test*		*Friability (%)*	*Hardness (Kg/cm2)*
	Average weight (mg)	*Deviation (%)*		
A	500.24	-0.00002	0.57	4.1
B	400.6	0	0.55	4.4
C	300.25	-0.00004	0.3	4.54
D	300.1	0	0.36	4.14
E	400.04	0.0014	0.61	4.54

Table 3: Results of disintegration, thickness, and loss on drying

Formulation	*Disintegration time (min)*	*Thickness (mm)*	*Loss on drying (%)*
A	15	5.83	6
B	24	5.90	4.5
C	20	4.76	8
D	18	5.86	8.1
E	30	5.94	7.2

Table 4: Data of extractive values

Formulation	*Alcohol soluble (%)*	*Chloroform soluble (%)*	*Water soluble (%)*
A	10	10	15
B	10	10	25
C	15	8	20
D	10	10	20
E	10	6	20

Table 5: Data of ash values

Formulation	Total ash value (%)	Sulphated ash value (%)
A	10.5	8.2
B	11	9
C	5.5	4.1
D	11	8.7
E	5	3.6

regular baseline rate following the subsequent administration of the drug. Otherwise, the response to the other drug may interfere with the outcomes.

Haemolytic Activity

On the day of the experiment, blood was taken out in heparin containing tubes from wistar/sprague Dawley rats and centrifugated at 1500 rpm for 5 minutes. Using a cold

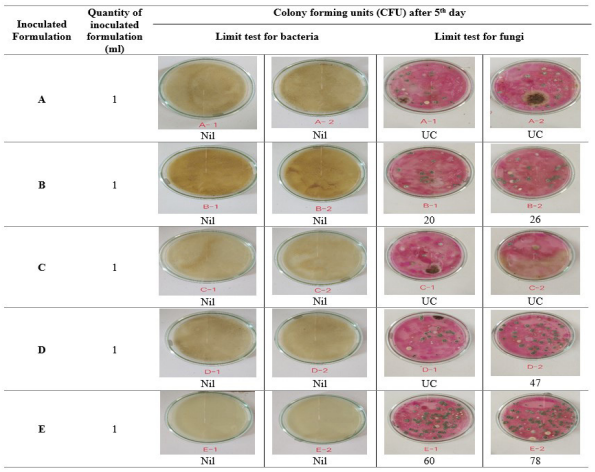

Figure 2: Microbial limit test for formulations A to E. **(Nil:** No growth of micro-organisms, UC: Uncountable)

phosphate buffer solution (PBS) pH 7.4, the pellet was washed for three times and then centrifugated at 1500 rpm for 5 minutes and resuspended in the same buffer. Different concentrations of selected formulation were prepared in the phosphate buffer solution pH 7.4 containing erythrocytes and further incubated for 60 minutes at 37°C in a shaking water bath. Photometric analysis at 540 nm was used to determine hemoglobin release after centrifugation (1500 rpm for 10 minutes). Whole hemolysis was attained using 0.1% triton X-100, yielding 100% control value. In experiments, less than 10 % hemolysis was considered as a non-toxic effect level. The triplicate readings were recorded.[12-14]

RESULTS AND DISCUSSION

Botanical Parameters

For physical evaluation, the macroscopy of formulations was studied by bearing in mind various parameters like taste, color, odor, and texture. The result of the microscopy of formulations is given in Table 1. The color of A, B, D tablets were found to be greenish, and C and E tablets shown brownish color. The odor of all tablets was found to be bitter and pungent because of the presence of alkaloids in all formulations. The taste of all tablets was found to be bitter because of the presence of alkaloids in all tablet formulations. The texture of all formulations was smooth, indicating all formulations were in good quality.

Physicochemical Parameters

Weight Variation Test

All formulations were showed a tablet weight more than 250 mg with a 5% maximum allowable difference. Also, all formulations pass the weight variation test as per the Indian pharmacopeial standard (Table 2).

Friability Test

The weight loss of tablets in percentage friability ranged from 0.30 to 0.70% means less than 1%, indicating that all formulations pass the friability test as per Indian pharmacopeial standard (Table 2).

Table 6: Phytochemical screening

Formulation	*Alkaloid test*				*Test for non-reducing sugar*
	Dragendorff's test	*Mayer's test*	*Wagner's test*	*Hager's test*	*Fehling's test*
A	Passes	Passes	Passes	Passes	Passes
B	Passes	Passes	Passes	Passes	Passes
C	Passes	Passes	Passes	Passes	Passes
D	Passes	Passes	Passes	Passes	Passes
E	Passes	Passes	Passes	Passes	Passes

Table 7: Result of antihypertensive activity

Formulation	*Dose (mg/kg)*	*Induced B.P. (mm/Hg)*	*B.P. after drug/sample (mm/Hg)*
Control	3.3	160-120	160-120
Standard	3.3	160-120	120-80
A	3.3	160-120	110-90
B	3.3	160-120	100-70
C	3.3	160-120	110-80
D	3.3	160-120	110-90
E	3.3	160-120	120-70

Table 8: Result of heavy metal test

Formulation	*Heavy metal test*		
	For bismuth	*For cadmium*	*For lead*
A	absent	absent	absent
B	absent	absent	absent
C	absent	absent	absent
D	absent	absent	absent
E	absent	absent	absent

Hardness Test

The hardness of tablets was in a range of 4 to 5 kg/cm^2 and showed appreciable hardness characteristics which facilitated its fast disintegration. Hence passes the hardness test as per Indian pharmacopeial standard (Table 2).

Disintegration Test

The disintegration time of selected tablets was between 15 and 30 minutes, which showed an appreciable and rapid disintegration (Table 3).

Thickness

The thickness of the tablets was found to be in 4 to 6 mm range. Hence passes the thickness test as per Indian pharmacopeial standard (Table 3).

Loss on Drying

All formulations showed loss of drying in the range of 4 to 8%, which follows quality attributes (Table 3).

Extractive Values

For evaluation of crude drugs, extractive values play a vital role. The alcohol-soluble extractive values of marketed formulations were in between 10 to 20%, chloroform soluble extractive values were in 6 to 10% range and water-soluble extractive values were in between 15 to 25% (Table 4). Lower extractive values designate adulteration, the addition of exhausted material, or incorrect processing during formulating, storage, or drying. The outcome of extractive values shown that there was no adulteration in any formulations.

Ash Values

In deciding the purity and quality of crude drugs in powdered form, ash values are helpful. High ash values are suggestive of adulteration or substitution contamination. The total ash values of formulations were in the 5 to 11% range (Table 5). The sulfated ash value of all formulations was observed to be in 4 to 10% in range (Table 5). Sulfated ash values were less as compared to total ash values.

Phytochemical Screening

The detailed examination of primary and secondary metabolites of the plant is part of a systematic and complete study of any crude drugs. Several qualitative chemical examinations are completed to create the profile of a given extract or fraction regarding the nature of the chemical composition. Only alkaloids and non-reducing sugar were present in all formulations (Table 6).

Pharmacological Parameters

Acute Toxicity Study

Acute toxicity study was conducted on wistar albino rats according to OECD 423 guidelines. As per guidelines, give 5, 50, 300, 2000 mg/kg body weight of rats. To give 1, 10, 60, 400 mg powder of herbal tablets was administered orally to the first experimental animal per body weight of wistar albino rats, respectively as per guidelines. In after dosing 14 days of observation is required in each dose. There was none side effect observed. So, the next 5000 mg/kg dose of herbal tablet was given to rats and observed under a certain period, so again, no side effects were observed. Finally, these herbal tablets showed no side effects up to 5000 mg/kg, indicating the selected tablet formulation was safe to treat hypertension.

Antihypertensive Activity

The invasive blood pressure was accurate and quantifiable. Individual variations were observed, the outcome of the trial product was compared with that of the standard drug. All herbal formulations were shown antihypertensive activity, but all formulations shown less values than the standard drug (Table 7). The standard drug shown accurate values because of purity of the standard compound. The all-herbal formulations showed fewer values because the mixture of phytoconstituents was present in all formulations.

Hemolytic Activity

Hemolytic activity of all formulations was within 5% hence there was no breakdown of red cells in the blood.

Toxicological Parameters

Limit Test for Bacteria

The micro organism count was found nil in all plates at 0–5 days (Figure 2). In the control plate, the micro-organism count was continuously increasing from 0–5 days.

Limit Test for Fungi

In all plates, the micro organisms count continuously increased from 0–5 days (Figure 2). In the control plate the count of micro organisms was continuously increasing from 0–5 days.

Heavy Metal Test

The heavy metal limit test was carried out to ensure product quality. Study resulted that heavy metals were absent in all formulations (Table 8). All formulations were free from metals and no adulteration was observed.

CONCLUSION

The study concluded that selected formulations pass most of the standardization parameters and can be recommended for human use. The present research work is in line with WHO requirements related with the standardization of herbal products which may provide the database related with standardization of the available marketed antihypertensive herbal formulations in India. This database will help in maintaining the quality of the products, which may be useful for empowering the regulatory procedure and curtailing quality breaches.

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