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Safety Evaluation of an Herbal Antiasthematic Preparation

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ABSTRACT

Medicinal plants have been used in various traditional systems, as they have potential against numerous diseases. Currently, more than 200 clinically useful prescription drugs are derived from plants. Most of these were developed because of their use in traditional medicine. In several ancient systems of medicine including Ayurveda, Siddha and Unani, Ipomoea aquatic, Cinnamomum zeylanicum and Piper longum, three medicinally important herbs from mainly Asian origin has vast number of therapeutic applications including antiasthematic activity but very little information is available about their safety profile.

It is a common believe that herbal products are safe because of which nobody care for the evaluation of their final preparation. We strongly recommend performing safety studies for the herbal preparations because all the extracts used are chemical in nature and there is strong possibility that these chemicals may react with each other and form some toxic compound or interact with the biomolecules. Keeping these things in mind, the present study has been envisaged and it was observed that water and chloroform extracts of Ipomoea aquatic, Cinnamomum zeylanicum and Piper longum are safe, based on the acute toxicity studies, as no abnormal changes are observed in treated animals. The combinations of both the extracts in 1:1:1 ratio are also safe and can be encapsulated into hard gelatin capsule of 000 size for human use.

Keywords: Ipomoea aquatic, Cinnamomum zeylanicum, Piper longum, Acute toxicity, Herbal extract, Antiasthamatic preparation

1. INTRODUCTION

The word Asthma is of Greek origin and means, "panting". The term Asthma is used to describe episodic shortness of breath. The first clinical description of asthma was made in 2nd century.[1] Asthma is a complex syndrome with many phenotypes in both adults and children. Its major characteristics include variable degree of airflow obstruction, bronchial hyperresponsiveness, and airway inflammation. For many patients, the disease has its root in infancy, and both genetic factors[1,2] and environmental factors such as viruses[3], allergens[4] and occupational exposures[5] contributes to its inception and evolution.

Unlike many diseases, which can be attributed to the life style of modern man, asthma is an ancient illness. Asthma is disease of the larger and medium sized airways of the lungs and there is obstruction to outflow of air from the lungs. Since, enough air does not reach the lungs for the exchange of gases, there is hurried breathing to compensate it. Cough is a frequent symptom in asthmatics. This occurs in order to throw out the excessive secretions produced in the lungs. This is particularly so in those who have respiratory infection as well. Cough gets relieved by the same measures as breathlessness. Bronchial asthma, commonly called asthma, consists of breathlessness and wheezing.

When the patient is not in an attack, he feels normal. When an asthma patient comes in contact with an allergic substance, it behaves as an antigen and reacts with the corresponding antibodies already present in his bodies. The Histamine and other substances liberated during the allergic reactions cause the following changes in the bronchi:

1. Bronchi muscles are constricted to the extent of lessening of the diameter (Calibre) of the bronchi.
2. Mucous membrane of the bronchi gets swollen, which further restricts the lumen of the bronchi.
3. Secretions are poured out from the swollen mucous lining into the constricted lumen of the bronchi.
4. When the bronchi are constricted and they are full of secretions, the patient has difficulty in breathing and his breath has a wheezing sound in it which is more on breathing out because then the bronchi get narrower.

Current therapeutic options in asthma consist of rescue, prophylaxis, and suppressive therapy. The currently accepted approach is to use drugs that suppress the inflammatory response as primary therapy thereby reducing the degree of bronchial hyperreactivity and improving long term control and outcome in asthma with the aim to decrease patient's baseline airways hyperreactivity and prevent it from increasing.[6,7]

The present status of antiasthamatic drugs from synthetic origin like beta receptor agonists (bronchdialators), steroids like corticosteroids, leukotrienes modifiers, other drugs like anti-inflammatory drugs and anti-cholinergic drugs, etc. are no safer drugs as they cause severe side effects like bleeding from stomach, loss of calcium from bones, mild headache, diarrohea, cataracts as in case of leukotriene receptor antagonist, fungal growth, transient loarsences and voice changes etc. as in case of steroid therapy, speeding heart, effect on skeletal muscle and tremor with long therapy and over dosage in case of beta receptor agonist drugs.[8]

Although having such large number of modern medicines, the health-care system has expanded and changed remarkably in recent years because of limited curative potential as well as adverse effects associated with current asthma therapy. So, patients with asthma are increasingly using complementary and alternative therapies. There are large numbers of medicinal plants that have been reported to possess anti-asthmatic effects.[9]

The importance of drugs from natural products is well established over the centuries. In developing countries of the world, there is still major reliance on crude preparation of plants used in traditional system of medicines for their primary healthcare. In countries such as India, the system of traditional medicines are particularly well developed and provide interesting new drug leads for potential development in western medicines.

WHO estimates that 80% of world population relies upon plants for primary medical treatment of common illness. But now the era of grand old system has probably passed out, it may be the time to develop a coherent approach to make use of new scientific age.

It is a common believe that these herbal products are safe because of which nobody care for the evaluation of their final preparation. Living organisms are an amalgamation of a plethora of chemicals. They interact with a wide variety of chemicals made by humans and nature. Our body is made of a number of complex biomolecules that are endlessly in contact with a variety of environmental chemicals. All chemicals have the ability to be toxic to a biological system. Indeed, there are no safe chemicals and only safe ways of using chemicals. In the biological system every cell functions in an organised manner. The chemicals cause their effect by interacting with cells to change the way the cells function. Understanding toxic potential of all the chemicals commonly used is very important for fixing safety levels. We strongly recommend performing safety studies for the herbal preparations because all the extracts used are chemical in nature and there is strong possibility that these chemicals may react with each other and form some toxic compound or interact with the biomolecules. Keeping these things in mind, the present study has been envisaged.

2. MATERIALS AND METHODS

2.1 Plant material

Plants used for antiasthematic activity were Ipomoea aquatic, Cinnamomum zeylanicum blume and Piper longum. The drug samples were washed thoroughly 2-3 times with running tap water and once with sterile water, air-dried, powdered and used for extraction.

2.2 Preparation of extracts

Fifty grams of each of the air-dried and coarsely powdered plant material was extracted with 200 ml each of chloroform and water using a soxhlet apparatus for 48 h. Extracts of plant materials either in water or chloroform are concentrated. The concentrated herbal extract, now in the form of a viscous liquid, is piped into a flow coater. The granulator sprays the concentrate onto minute particles of a base material as the moisture slowly dissipates. With the moisture evaporated, the granules are dry and ready to be packaged. These granules were stored at 4^{o}C in airtight bottles for further investigations. The granules can then be pressed into easy-to-swallow tablets or encapsulated.

2.3 Preparation of Formulation

Three hundred thirty milligram granules of each plant extract was encapsulated into hard gelatin capsule of 000 size.

2.4 Animals

Swiss male mice (25 - 30 g), randomly bred and maintained in the SriRam College of Pharmacy, Banmore animal facility were used for the study. The animals were housed in polypropylene cages on dust free rice husk as the bedding material, and were provided with pellet diet (Amrut Ltd., India) and water ad libitum. The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). The animals are grouped into following groups:

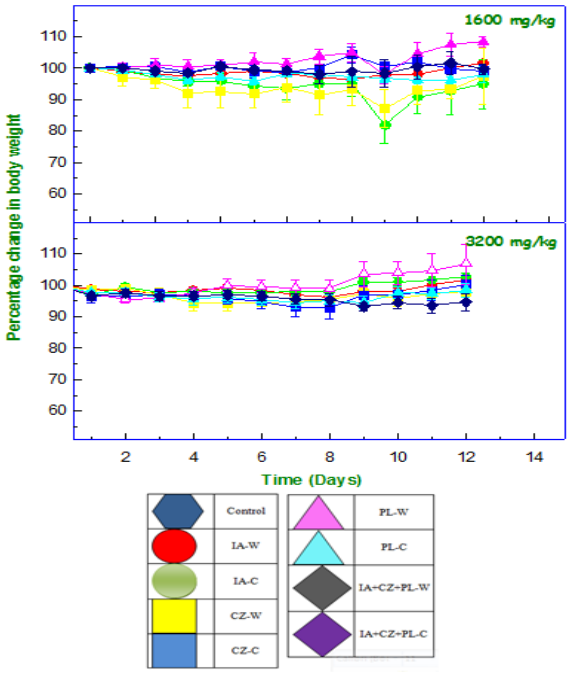

Figure 1: Percentage Change in Body Weight (Oral LD_{50})

2.5 Acute toxicity test

The water extracts were reconstituted in water and the chloroform extracts are reconstituted in DMSO. Two doses of the extracts 1600 mg/kg and 3200 mg/kg were administered to the mice. For each dose and extract a group of four mice were used. Food and water were withheld two hours prior to and two hours post oral administration. The animals were weighed daily, and food and water consumption were recorded. They were also observed for general behaviour and mortality for 14 days. After 14 days the mice were anaesthetised with ether and blood was withdrawn from orbital plexus for various haematological and biochemical analysis. They were sacrificed by cervical dislocation and vital organs were removed, weighed and preserved.

2.6 Clinical Pathology

All the animals were fasted overnight on day 14 at the termination of study. Blood samples were collected by puncturing the orbital plexus with the help of a fine heparinised glass capillary tube under light ether anaesthesia in heparinised vials (0.5 ml) for haematology and rest of the blood was centrifuged to separate plasma for clinical biochemistry.

2.7 Haematology

Analysis of hematological variables viz., RBC count, WBC count and Hb concentrations were carried out using a Autoanalyser.

2.8 Clinical biochemistry

Biochemical variables were analysed in plasma using Diagnostic kits of Merk (India) Ltd., Mumbai and using Semi-Auto Analyser.

Table 1: Change in Haematological variables

Group	Dose mg/kg	WBC (x 10^3/µl)	RBC (x 10^6/µl)	Hgb (g/dl)
Control	0	20.5±3.8	8.3±0.4	12.4±0.7
IA-W	1600	18.2±3.4	8.9±0.7	13.9±0.9
	3200	19.9±1.7	8.4±0.1	13.5±0.1
IA-C	1600	21.0±4.8	8.5±0.4	13.1±0.3
	3200	19.9±1.8	7.9±1.4	12.4±1.3
CZ-W	1600	18.2±2.3	9.0±0.4	13.6±0.2
	3200	22.4±1.6	8.7±0.2	14.0±0.5
CZ-C	1600	22.1±4.8	9.5±0.3	15.1±0.3
	3200	18.9±2.1	7.4±1.3	11.4±2.3
PL-W	1600	18.3±4.6	9.1±0.9	14.1±1.4
	3200	20.9±2.0	8.8±0.1	13.7±0.1
PL-C	1600	16.1±2.9	9.2±0.2	12.95±0.3
	3200	12.2±2.9	7.7±0.2	11.9±0.5
IA+CZ+PL-W	1600	14.8±1.7	7.8±0.2	11.7±0.6
	3200	15.1±1.6	8.5±0.3	12.5±0.4
IA+CZ+PL-C	1600	15.2±2.5	7.8±0.2	11.7±0.5
	3200	15.8±2.7	7.9±0.5	12.2±0.7
P		NS	NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control. NS- No significant difference from control

Table 2: Change in biochemical parameters

Group	Dose mg/kg	GLU (mg/dl)	CHOLES (mg/dl)	TP (g/dl)	UREA (mg/dl)	BILIRUBIN (mg/dl)	CRETAINNE (mg/dl)
Control	0	246.1±5.3	99.8±2.3	5.4±0.25	27.32.6±	0.47±0.08	0.24±0.02
IA-W	1600	216.1±15.9	83.7±8.4	6.4±0.6	26.9±1.3	1.01±0.64	0.27±0.06
	3200	189.1±17.4	86.5±12.0	5.7±0.5	30.8±2.1	0.56±0.18	0.32±0.06
IA-C	1600	223.9±19.6	86.5±9.6	5.9±0.8	31.6±1.8	0.49±0.15	0.37±0.04
	3200	188.3±18.2	110.8±8.7	6.8±0.4	33.8±1.9	0.50±0.15	0.25±0.05
CZ-W	1600	242.2±5.5	112.1±11.5	8.7±1.0	27.0±12.8	0.35±0.71	0.34±0.07
	3200	253.2±15.4	108.7±12.5	5.9±0.3	35.2±2.1	0.49±0.06	0.34±0.07
CZ-C	1600	222.9±9.6	86.5±9.7	7.9±0.8	39.6±2.8	0.47±0.05	0.47±0.04
	3200	198.3±28.2	110.8±2.7	6.3±0.4	40.8±2.9	0.51±0.05	0.26±0.04
PL-W	1600	210.1±35.9	84.7±9.4	7.4±0.6	26.9±1.0	1.00±0.24	0.37±0.06
	3200	183.1±19.4	86.5±16.0	6.7±0.5	32.8±3.1	0.58±0.08	0.40±0.05
PL-C	1600	214.4±19.4	88.4±13.5	7.1±0.3	26.7±6.7	0.46±0.01	0.37±0.02
	3200	220.9±10.2	96.1±3.4	5.3±0.3	30.2±2.3	0.32±0.07	0.28±0.03
IA+CZ+PL-W	1600	205.2±12.2	100.3±5.4	7.5±0.4	36.4±2.6	0.67±0.08	0.44±0.03
	3200	262.2±48.9	139.0±22.5	7.1±0.9	38.6±4.8	1.22±0.39	0.32±0.05
IA+CZ+PL-C	1600	239.3±8.6	108.3±7.5	6.9±0.4	36.9±4.1	0.43±0.06	0.39±0.05
	3200	243.8±25.9	111.2±10.3	6.2±0.2	39.4±6.5	0.42±0.10	0.21±0.01
P		<0.001		NS		NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control. NS- No significant difference from control

Table 3: Change in enzymes

Group	Dose mg/kg	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)	ALKP (IU/L)
Control	0	120.5±5.6	48.6±5.3	785.0±4.2	246.1±3.6
IA-W	1600	118.5±10.2	32.9±12.86	741.3±25.6	242.2±13.3
	3200	111.4±19.8	55.3±5.1	774.2±151.5	252.6±18.1
IA-C	1600	117.9±13.9	57.2±11.6	909.5±58.8	242.3±17.2
	3200	118.5±12.5	53.7±7.4	738.1±154.2	248.2±17.9
CZ-W	1600	157.9±4.4	82.2±4.7	607.1±23.5	215.9±8.3
	3200	107.8±7.1	31.0±3.2	784.7±42.9	181.8±61.2
CZ-C	1600	107.9±23.9	67.2±11.7	909.5±58.8	262.3±27.2
	3200	82.5±15.5	43.7±1.5	708.1±154.2	238.4±27.5
PL-W	1600	108.5±12.2	33.9±14.8	731.3±27.6	241.2±23.3
	3200	111.4±19.8	55.3±5.1	574.2±151.5	282.6±38.1
PL-C	1600	119.8±10.5	58.9±13.3	681.0±19.9	299.4±8.1
	3200	103.9±9.1	32.2±0.2	999.1±68.7	190.49±34.1
IA+CZ+PL-W	1600	143.9±27.3	81.0±4.6	984.0±53.6	261.4±19.1
	3200	217.2±49.1	36.7±9.1	795.9±26.0	125.0±26.1
IA+CZ+PL-C	1600	143.4±37.7	73.2±22.3	749.8±35.7	235.1±17.6
	3200	125.9±25.8	26.3±3.4	710.0±77.0	148.3±27.7
P		NS	NS	<0.05	<0.05

Values are Mean ±SEM (n=4); * Significantly different from control. NS- No significant difference from control

Table 4: Change in Organ/Body Weight Index (OBI)

Group	Dose mg/kg	LIVER	SPLEEN	KIDNEY	LUNGS	HEART
Control	0	5.13±0.17	0.49±0.01	1.41±0.00	0.63±0.03	0.57±0.02
IA-W	1600	5.24±0.10	0.56±0.04	1.49±0.20	0.71±0.01	0.61±0.06
	3200	6.03±0.30	0.65±0.03	1.52±0.12	0.72±0.01	0.58±0.04
IA-C	1600	5.21±0.09	0.52±0.22	1.61±0.90	0.67±0.36	0.67±0.36
	3200	6.02±0.30	0.64±0.10	1.69±0.12	0.69±0.13	0.69±0.03
CZ-W	1600	6.24±0.10	0.58±0.05	1.69±0.20	0.71±0.01	0.64±0.06
	3200	6.03±0.40	0.66±0.01	1.47±0.13	0.72±0.01	0.58±0.04
CZ-C	1600	5.97±0.70	0.54±0.12	1.82±0.10	0.72±0.14	0.65±0.02
	3200	6.19±0.09	0.80±0.15	1.98±0.23	0.65±0.12	0.56±0.02
PL-W	1600	5.68±0.30	0.61±0.07	1.52±0.18	0.81±0.11	0.61±0.01
	3200	4.96±0.16	0.48±0.07	1.62±0.12	0.62±0.02	0.58±0.04
PL-C	1600	5.11±0.09	0.92±0.32	1.51±0.97	0.67±0.36	0.87±0.36
	3200	6.12±0.60	0.64±0.17	1.84±0.12	0.74±0.13	0.54±0.03
IA+CZ +PL-W	1600	6.58±0.70	0.78±0.12	1.86±0.25	0.85±0.08	0.62±0.04
	3200	4.71±0.15	0.47±0.03	1.42±0.16	0.64±0.06	0.64±0.14
IA+CZ +PL-C	1600	6.09±0.55	2.04±1.47	1.64±2.86	0.72±2.22	0.60±1.03
	3200	5.39±0.27	0.87±0.21	1.71±0.13	0.65±0.046	0.55±0.05
P		NS	NS	NS	NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control. NS- No significant difference from control

3. RESULT & DISCUSSION

Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive.[10] In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. An upward trend has been observed in the research on herbals. However, proper methodologies for the research and development are the need of the day for tapping the full therapeutic potentials of plants. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species.[11] According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care.[12] Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants.[13] In recent time there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day.[14]

In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses. It, therefore, become essential for a researcher to do the safety studies of the extract in the initial stage of drug discovery. In several ancient systems of medicine including Ayurveda, Siddha and Unani, Ipomoea aquatic, Cinnamomum zeylanicum blume and Piper longum, three medicinally important herb from mainly Asian origin has vast number of therapeutic applications including antiasthematic activity but very little information is available about their safety profile.[15-17]

In the safety evaluation of a test substance, determination of acute toxicity is generally the initial step and provides information on health hazards that may arise from an acute exposure by the intended route of exposure. The experimental protocols recommended by different authorities worldwide place a great deal of emphasis on effects of test substance on mortality of treated animals.[18] As per fixed dose procedure, the selection of appropriate dose levels during acute toxicity testing and final classification of the test material depends on lethality/evident toxicity signs observed during the experiment. The present study was carried out to assess extracts of Ipomoea aquatic, Cinnamomum zeylanicum blume and Piper longum for their acute toxic potential by fixed dose procedure adopted by the Organisation for Economic Co-operation and Development (OECD).[19] This approach avoids using death of

animals as an endpoint and relies instead on the observation of clear signs of toxicity after administration of test material at a series of fixed dose levels. The method also renders information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS). In the present study, the test substances were administered orally in different doses viz., 1600 and 3200 mg/kg body weight to four animals in each group. The treated animals were observed for mortality, untoward clinical/toxic signs, alterations in body weight gain and necropsy findings during the study.

The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the tested dose levels for all the products. No mortality was observed in any of the groups and doses. The estimated LD_{50} is more than 3.2 g/kg for all the extracts by the oral route. There was no significant change in body weights of treated and control groups (Figure 1). At the termination of study, blood samples were collected by puncturing the orbital plexus with the help of a fine heparinised glass capillary tube under light ether anaesthesia in heparinised vials (0.5 ml) for haematology and rest of the blood was centrifuged to separate plasma for clinical biochemistry. There was no significant change in any of the haematological variables viz., RBC, WBC and Haemoglobin compared to the control (Table 1). There was also no significant change in the biochemical variables and enzymes levels (Table 2 and 3).

The toxic nature of the administered product is generally correlated with its ability to produce a 10% or more decrement in body weight or growth rate of the selected test animals.[20] From the results of the current study, the overall percent body weight gain in mice treated was found to be normal at the end of 14 days. Postmortem toxicology of treated animals is customarily recommended in the adopted guidelines for acute toxicity testing.[21] The gross pathological finding for each animal is genuinely considered as potential source of information on the target organ/system and the toxic nature of the chosen test substance. Necropsy examination conducted at the termination of 14 day observation study was normal for all the animals and did not show any significant treatment related macroscopic changes of organs or other structures. Absolute weights of the organs i.e. lungs, liver, kidneys, spleen, testis/ovaries and heart were recorded immediately after dissection of all animals at the end of the study period. Body-Organ weight Indices (BOI) was calculated. There were no significant difference in the BOI when compared to control animals (Table 4).

4. CONCLUSION

The water and chloroform extracts of Ipomoea aquatic, Cinnamomum zeylanicum blume and Piper longum are safe, based on the acute toxicity studies, as no abnormal changes are observed in treated animals. The combinations of all the extracts in 1:1:1 ratio are also safe and can be encapsulated into hard gelatin capsule of 000 size for human use.

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