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Acute Toxicity Study of an Herbal Hypolipidemic Preparation

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ABSTRACT

Medicinal plants have been used in various traditional systems, as they have potential against numerous diseases like cardiovascular disease. 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, Achyranthus aspera & Murraya koenigii, two medicinally important herb from mainly Asian origin has vast number of therapeutic applications including hypolipidemic 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 Achyranthus aspera & Murraya koenigii 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 ratio are also safe and can be encapsulated into hard gelatin capsule of 000 size for human use.

Key words: Achyranthus aspera, Murraya koenigii, Acute toxicity, Herbal extract, Hypolipidemic preparation

1. INTRODUCTION

Hyperlipoproteinemias cause atherosclerosis, which is a major cause of death in the developed world and is also now becoming a major cause of morbidity not only in India but it has its tentacles globally. Diabetes mellitus is a disease characterized by hyperglycaemia and hyperlipidaemia which leads to an increased risk of atherosclerosis and other cardiovascular diseases.[1]

WHO predicts that India will have 60% of the world heart patients in future. This situation puts forth a challenge of the new millennium in front of us. The American heart association has identified the primary risk factor associated with atherosclerosis as evaluated levels of total cholesterol and triglyceride in the blood.[2] In India, heart diseases are not related to a particular class of people. They are spread over all the segments of society. Change in life style, high stress level, high fat diet, high serum lipid levels, hypertension, increased environmental pollution and adulteration in food causing excessive formation of free radicals are responsible for initiation of hyperlipidemia and atherosclerosis. According to the cardiological society of India, 50 million people in India suffer from heart diseases, this figure warns us about the seriousness of the disease.

Atherosclerosis has spread all over the world. In the developing countries major portion of the budget is spent on the health care system. Due to the seriousness nature of heart diseases it is very essential and urgent to fight against this disease in a systematic manner . Atherosclerosis referred to as a silent killer is one ot the leading causes of death in the developed countries and is on the rise in developing countries like India.[3]

Epidemiological studies have established a direct relationship with serum cholesterol and coronary artery diseases. High levels of total cholesterol and, more importantly, LDL cholesterol are major coronary risk factors (National Cholesterol Education Program, 1994) and triglycerides are also independently related to coronary artery disease. Several studies show that an increase in HDL cholesterol is associated with a decrease in coronary risk.

Atherosclerosis, for which no effective drugs are found till today in the modern system of medicine. None of the existing hyperlipoproteinemic drugs are fully effective, safe and totally free of side effect. Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects.[4]

Medicinal plants have been used in various traditional systems, as they have potential against numerous diseases like cardiovascular disease. Currently, more than 200 clinically useful prescription drugs are derived from plants. Most of these were developed because of their use in traditional medicine. India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plant. In the present scenario, the demand for herbal products is growing exponentially through out the world and major research institutions are currently conducting extensive research on plant material for their potential medicinal value.

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 hypolipidemic activity were Achyranthus aspera & Murraya koenigii. The leaf 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 4oC in airtight bottles for further investigations. The granules can then be pressed into easy-to-swallow tablets or encapsulated.

2.3 Preparation of Formulation

Five hundred 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 Sri Ram 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.

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.

2.6.1 Haematology

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

2.6.2 Clinical biochemistry

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

3. RESULT & DISCUSSION

Knowledge of herbs has been handed down from generation to generation for thousands of years.[5] 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.[6] 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. 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.[7] According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care.[8] Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants.[9] 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.[10]

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, Achyranthus aspera & Murraya koenigii, two medicinally important herb from mainly Asian origin has vast number of therapeutic applications including hypolipidemic activity[11,12] but very little information is available about their safety profile.

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.[13] 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 Achyranthus aspera & Murraya koenigii for their acute toxic potential by fixed dose procedure adopted by the Organisation for Economic Co-operation and Development (OECD).[14] 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 LD50 is more than 3.2 g/kg for all the extracts by the oral route. Oral administration of AA-C showed a significant decrease in body weight one day after administration, otherwise there was only a significant increase in body weight in few groups 7 and 14 days post administration compared to the control (Table 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 2). There was also no significant change in the biochemical variables and enzymes levels (Table 3 and 4).

Table 1: Change in Percentage body weight

Group	Dose mg/kg	Day 1	Day 7	Day 14
Control	0	102.0±1.19	100.49±1.19	99.7±1.09
AA-W	1600	106.0±1.19	116.0±3.4*	121.4±3.8*
	3200	96.5±1.89	99.9±1.28	104.2±3.4
AA-C	1600	103.4±1.49	115.9±2.41	127.2±4.71*
	3200	92.8±3.3	98.9±1.61	104.7±3.0
MK-W	1600	105.8±1.0	114.19±3.2*	124.3±4.4*
	3200	95.1±2.39	98.3±5.49	99.9±4.5
MK-C	1600	103.5±1.69	111.8±3.9	118.9±5.1*
	3200	103.4±5.7	111.2±3.5	117.9±5.5*
MK+AA-W	1600	102.7±1.8	112.0±2.9	118.9±6.3*
	3200	98.8±2.2	100.9±2.8	106.69±3.0
MK+AA-C	1600	102.5±2.3	115.1±3.3*	124.9±5.1
	3200	94.2±2.3	99.9±4.9	104.8±4.3
F		3.33	4.85	4.79
P		<0.01	<0.001	<0.001

Values are Mean ±SEM (n=4); * Significantly different from control.

Table 2: Change in Haematological variables

Group	Dose mg/kg	RBC($x10^3$cells/µl)	WBC ($x10^3$cells/µl)	Hb (g/dl)
Control	0	8.0±1.0	12.2±1.9	11.5±0.9
AA-W	1600	9.3±0.4	10.1±0.8	13.7±0.9
	3200	9.3±0.3	14.9±3.3	13.6±0.9
AA-C	1600	8.9±0.3	14.4±0.9	12.2±0.6
	3200	8.9±0.3	10.3±0.8	13.1±0.6
MK-W	1600	9.0±0.4	8.9±1.3	13.4±0.3
	3200	8.2±0.4	9.9±2.5	12.0±1.3
MK-C	1600	8.6±0.4	10.3±0.9	12.8±0.7
	3200	7.9±0.5	18.7±3.3	11.7±1.1
MK+AA-W	1600	9.9±0.3	10.8±2.4	14.5±0.6
	3200	8.5±0.5	15.8±2.4	11.9±1.0
MK+AA-C	1600	8.9±0.3	11.8±1.4	12.9±0.8
	3200	8.9±0.6	14.0±1.8	12.4±0.9
F		1.84	1.72	0.92
P		NS	NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control.

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.[15] 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.[16]

Table 3: Change in biochemical parameters

Group	Dose mg/kg	Glucose (mg/dl)	Urea(mg/dl)	TP (g/dl)	Cholestrol (mg/dl)
Control	0	222.4±9.1	48.7±3.2	5.7±0.2	118.3±8.9
AA-W	1600	164.9±9.9*	57.0±4.9	6.3±0.2	92.1±10.1
	3200	221.9±13.1	48.6±5.9	6.6±0.3	132.1±14.9
AA-C	1600	161.9±9.9*	51.7±5.3	6.2±0.8	89.0±2.9
	3200	188.9±14.1	51.6±5.3	5.3±0.2	104.9±16.9
MK-W	1600	167.9±5.9*	52.1±2.8	6.4±0.3	108.9±9.9
	3200	179.9±7.9*	49.6±7.7	5.9±0.3	135.1±19.9
MK-C	1600	134.1±11.9*	45.5±5.3	7.3±0.3	114.9±18.9
	3200	191.1±10.9	50.1±7.6	6.6±0.6	120.9±12.1
MK+AA-W	1600	161.1±2.9*	51.1±3.5	6.3±0.4	108.99±14.1
	3200	207.1±8.9	57.9±13.9	6.7±0.7	114.9±5.9
MK+AA-C	1600	191.9±12.9	54.3±2.8	6.2±0.2	102.1±2.9
	3200	198.1±17.1	47.0±10.1	6.0±0.4	100.9±12.1
F		5.90	0.3	0.97	0.95
P		<0.001	NS	NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control.

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 5).

Table 4: Change in enzymes

Group	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	LDH (IU/L)	ALKP (IU/L)
Control	0	48.8±5.5	77.1±4.0	785.0±48.2	525.2±49.2
AA-W	1600	42.9±2.5	77.9±3.0	731.3±27.6	468.8±79.9
	3200	40.2±5.9	79.9±4.3	708.1±154.2	310.8±84.3
AA-C	1600	42.2±4.0	72.0±5.5	681.0±19.9	606.2±89.9
	3200	49.9±4.1	75.9±5.0	574.2±151.5	372.4±79.9
MK-W	1600	40.0±5.0	73.3±9.0	607.1±23.5	436.1±336.9
	3200	37.0±4.0	66.7±6.0	784.7±42.9	328.9±89.9
MK-C	1600	40.0±2.0	64.4±5.6	809.5±58.8	459.9±89.8
	3200	45.1±1.0	66.9±5.0	795.9±26.0	330.2±89.8
MK+AA-W	1600	42.1±4.0	76.1±12.0	749.8±35.7	488.1±79.9
	3200	42.0±5.5	81.9±8.9	710.0±77.0	279.2±79.1
MK+AA-C	1600	52.5±12.2	89.0±14.9	884.0±53.6	524.1±73.0
	3200	44.2±3.3	87.9±5.9	899.1±68.7	333.9±61.3
F		0.70	0.85	3.2	2.22
P		NS	NS	<0.05	<0.05

Values are Mean ±SEM (n=4); * Significantly different from control.

4. CONCLUSION

The water and chloroform extracts of Achyranthus aspera & Murraya koenigii 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 ratio are also safe and can be encapsulated into hard gelatin capsule of 000 size for human use.

Table 5: Change in Organ/Body Weight Index (OBI)

Group	Dose mg/kg	Liver	Spleen	Kidney	Heart	Lungs
Control	0	5.99±0.51	0.56±0.04	1.25±0.08	0.54±0.08	0.67±0.04
AA-W	1600	5.38±0.32	0.49±0.08	1.24±0.17	0.55±0.07	5.39±0.29
	3200	5.54±0.31	0.50±0.14	1.37±0.04	0.59±0.06	5.54±0.30
AA-C	1600	5.61±0.24	0.55±0.10	1.41±0.09	0.58±0.03	5.61±0.11
	3200	5.99±0.22	0.55±0.09	1.41±0.09	0.63±0.09	5.99±0.15
MK-W	1600	5.67±0.26	0.57±0.03	1.35±0.03	0.52±0.06	5.65±0.16
	3200	5.31±0.27	0.59±0.14	1.32±0.12	0.60±0.06	5.32±0.20
MK-C	1600	5.33±0.24	0.52±0.04	1.30±0.02	0.60±0.03	5.13±0.19
	3200	5.95±0.39	0.62±0.19	1.30±0.05	0.65±0.05	5.95±0.31
MK+AA-W	1600	5.49±0.34	0.50±0.05	1.25±0.04	0.61±0.10	5.47±0.30
	3200	5.92±0.42	0.58±0.10	1.30±0.03	0.69±0.06	5.99±0.38
MK+AA-C	1600	5.25±0.25	0.55±0.03	1.36±0.10	0.49±0.03	5.24±0.10
	3200	5.99±0.33	0.78±0.44	1.48±0.05	0.65±0.05	5.98±0.40
F		0.98	0.57	0.99	0.97	0.64
P		NS	NS	NS	NS	NS

Values are Mean ±SEM (n=4); * Significantly different from control.

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