Acute and Sub-acute Toxicity of Amalakyadi Churna

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Summary

The acute and sub-acute toxicity of ethanolic extract of Amalakyadi churna (AC) was undertaken to assess its safety and tolerability profile in long term treatment. AC is one of the Ayurvedic formulations, widely used as carminative, appetizer, purgative etc. Acute toxicity (72-hours) and sub-acute toxicity (30-days) studies with AC were done on Swiss albino mice to determine its consequences on morphological, body-weight, organ-weight changes, histopathological, biochemical, mutational, hematological variations and mortality. Acute toxicity determination indicated that, AC is a moderatively toxic (LD_{50} =313mg/kg b.wt. ip). The sub-acute toxicity studies of AC (25 and 40mg/kg b.wt for 30 days ip.) did not induce any significant changes in different parameters studied, except a mild hematotoxic effect and lowering lipid metabolism. The results of the present study strongly suggested that, there was increase in the drug tolerance in mice by dose fractionation and wide margin of safety at its therapeutic dose levels.

Key words: Amalakyadi churna; Acute toxicity; Sub-acute toxicity.

Introduction

Amalakyadi churna consists of four different plant ingredients of fruits of *Phyllanthus emblica* L (Phyllanthaceae), *Piper longum* L (Piperaceae), *Terminalia chebula* Retz (Combretaceae), roots of *Plumbago zeylanica* L (Plumbaginaceae). All these, herbal drugs are extensively used in traditional system of medicine and by folk practitioners in India, because of their remarkable therapeutic potential, few of them have been already explored scientifically through *in vitro* and *in vivo* studies (1-3), also explored presence of many active principles for various pharmacological actions (4-5).

Though, the medicinal plants are considered to be non-toxic, the sustenance of life, can be toxic, if consumed too much. Thus a key aspect to understanding the toxicity of materials is to know how much of a substance-the dose can cause harm regarding safety and efficacy. Hence, the scientific approach through experimental and clinical validation of efficacy and documentation of useful herbs, herbal preparations and other formulations is necessary, as is done in modern medicine, animal toxicity studies are also required to establish the potential adverse effects.

But there is a strong evidence for mechanism of action in scientific and clinical trials of the Amalakyadi churna is lacking. Hence, the present study was undertaken to evaluate the detailed acute and sub-acute toxicity of Amalakyadi churna to assess its safety and tolerability profile in long term treatment.

Materials and Methods

Amalakyadi Churna: Amalakyadi churna was prepared by using standard formulation of an ancient Indian Physician Sarangadhara (1300 AD ~ 1400AD) (6). The pre-cleaned dried fruits of *Phyllanthus emblica* L., *Piper longum* L., *Terminalia chebula* Retz., roots of *Plumbago zeylanica* L. and rock salt were powdered separately and mixed them in equal proportions.

The 100g of Amalakyadi churna was extracted with 90% ethanol at 50 -60°C in a soxhlet apparatus. The extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (50 -60°C). The dried extract was used to evaluate toxicity studies.

Preparation of Drug: The dried 90% of the ethanolic extract of AC was taken, weighed exactly in required dose in mg/kg b.wt and was dissolved in 10ml of distilled water.

Animals: The animal care and handling were done according to the guidelines set by the World Health Organization (WHO). Random bred 6-8 week-old Swiss albino mice from our animal colony weighing 25±2g were used. The animals are bred under controlled conditions of temperature (23±2°C), humidity (50±5%) and light (10h and 14 h of light and dark, respectively). and are maintained on standard sterile mouse food and water adlibitum. 5-6 animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment.

Acute Toxicity Studies: The healthy, adult Laboratory bred Swiss albino mice of either sex were selected and randomly distributed into ten different groups of 10 animals each. The animals were fasted overnight. The 1st group was given double distilled water (0.2ml/mouse), treated as control. The 2nd, 3rd, 4th, 5th and 6th groups were received single dose of AC extract at 200, 250, 300, 350 and 400mg/kg b.wt. intraperitoneally, respectively. Where as 7th, 8th, 9th and 10th groups were given orally at 500mg, 1g, 2g and 3g/kg b.wt. respectively. All treated mice were observed continuously for first 6 hours and the LD_{50} value was determined in 72 hours (7).

Sub-acute Toxicity Study: The albino mice of either sex weighing 18-22g were assigned to 4 groups (5 per each group), Group-I were kept untreated, served as control, Group-II received distilled water as vehicle control group. Group-III and IV received 25mg/kg and 40mg/kg b.wt. corresponding to 1/12th and 1/8th respectively of the LD_{50} of AC ip for 30 days. Body weight, food and water intake were monitored daily. The 15 and 30 days of post treatment period, blood was collected and subjected for hematological profile (8). All the animals were sacrificed on 31st post treatment day, femoral bone marrow cells and other vital organs were collected for detailed evaluation of mutational (9), organ weight (10) biochemical (11) and histopathological changes (12)

Statistical analysis: All the data are expressed as mean ± S.E.M. (standard error of the mean). Significance level in different groups were analyzed using student't' test. P values less than 0.05 were considered to be statistically significant.

Results and Discussion

Acute toxicity: The LD_{50} of AC was found to be 313mg/kg b wt. intraperitoneally. At acute dose of 400mg/kg b.wt of the drug dose and above the animals posed toxic symptoms like weight loss, reduced food and water intake, poor response to external stimuli, drowsiness, lethargy and all animals were died with in 72 hours. At acute doses 350, 300 and 250mg/kg b.wt. of the drug also

expressed similar toxic symptoms, and observed the mortality rate as 80%, 40% and 20% respectively. Up-to the acute dose of 200mg/kg b.wt. ip of AC was tolerated by mice without any apparent adverse manifestations (Chart-1). The drug is practically non-toxic at oral doses, it was confirmed through by administration of the drug up to 3g/kg b.wt. orally, did not poses any threat or toxic symptoms as compared to intraperitoneal administration. This may be because the drug was absorbed by the gastrointestinal tract or it might have detoxified by the liver. The acute toxicity studies of Amalakyadi churna indicates it has moderate toxicity. According to the classification, the moderate toxicity of drug is between 50 to 500mg/kg b.wt. (13).

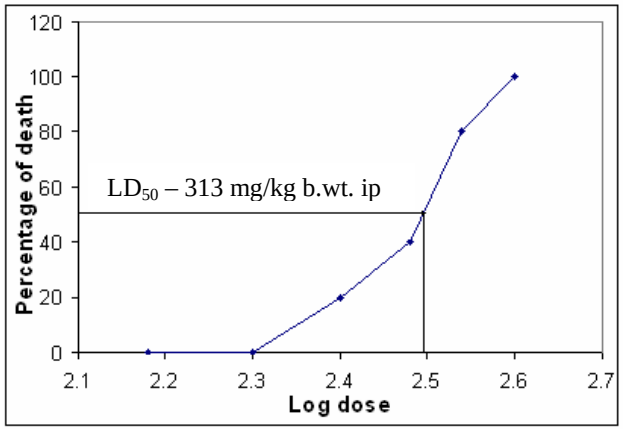

Chart -1 Acute intraperitoneal toxicity for 72 hours mortality of Amalakyadi churna in Swiss Albino mice

Subacute toxicity studies: General symptoms and Body weights: At 25 and 40 mg/kg b.wt of Amalakyadi churna intraperitoneally did not show any external manifestation of toxic symptoms and other behavioral patterns, body weights, no mortality was recorded even after 30 days of drug administration and there by the drug was proved to be safe up to this level.

Organ Weights: The macroscopic examination of the major organs such as lungs, liver, kidney, stomach, intestine, pancreas, adrenals and heart did not show any gross changes in their appearance and size, but considerable enlargement of spleen and reduction in the weight of the lungs was observed indicating some toxic inflammatory reactions induced by the AC treatment (Table-1).

Table –1 Organ weight in albino mice after 30 days of treatment with Amalakyadi churna (Organ weight expressed in g/100g body weight)

Organs	**Sexes**	**Vehicle Control** (Distilled Water)	**25 mg/kg drug**	**40 mg/kg drug**
Liver	Male	6.218 ±0.545	6.06 ± 1.414	5.969 ± 0.680
	Female	5.975 ± 0.246	6.03 ± 0.706	6.264 ± 0.180
Spleen	Male	0.548 ± 0.060	0.568 ±0.141	0.572 ± 0.350
	Female	0.645 ± 0.001	0.633 ± 0.448	0.958 ± 0.020 **a**
Lung	Male	0.736 ± 0.002	0.716 ± 0.392	0.714 ± 0.090
	Female	0.738 ± 0.071	0.755 ± 0.035	0.719 ± 0.010
Kidney	Male	1.805 ± 0.082	1.841 ± 0.170	1.861 ± 0.014
	Female	1.593 ± 0.707	1.564 ± 0.720	1.315 ± 0.007
Adrenals	Male	0.047 ± 0.004	0.048 ± 0.010	0.045 ± 0.032
	Female	0.038 ± 0.003	0.039 ± 0.005	0.038 ± 0.028
Stomach	Male	1.450 ± 0.710	1.551 ± 0.439	1.609 ± 0.029
	Female	1.202 ± 0.018	1.371 ± 0.709	1.391 ± 0.085
Heart	Male	0.617 ± 0.053	0.626 ± 0.042	0.618 ± 0.043
	Female	0.552 ± 0.020	0.549 ± 0.008	0.546 ± 0.015
Pancreas	Male	0.503 ± 0.387	0.522 ± 0.071	0.466 ± 0.046
	Female	0.526 ± 0.009	0.535 ± 0.014	0.419 ± 0.08
Intestine	Male	13.25 ± 0.179	13.60 ± 0.430	13.099± 1.65
	Female	13.14 ± 0.102	13.37 ± 0.440	12.821± 0.58
Thymus	Male	0.040 ± 0.009	0.041 ± 0.003	0.043 ±0.00
	Female	0.038 ± 0.008	0.038 ± 0.008	0.042 ±0.00

Note: a= statistically significant (>0.05%) when compared the treated groups of mice with non-drug treated vehicle control group.

Hematological Studies: The 30 days administration of AC has shown slight increase in the total number RBC cells but the total count of WBC and clotting time of blood remained same (Table – 2). The increase in the RBC count also influences the simultaneous elevation in the hemoglobin level, this increase in the hemoglobin level might be due to the increased absorption of iron by vitamin –C

(chief constituent of fruits of *P. emblica*), and this plant is one of the important constituent of Amalakyadi churna. There was slight increase in the number of neutrophils and decrease in the percentage of lymphocyte cells, but these changes were statistically insignificant (Table-3). The similar earlier reports suggested that the enlargement of spleen and increase in the neutrophils counts indicate the toxic inflammatory reactions (14).

Table –2 The peripheral blood changes in mice treated for 15 and 30 days with Amalakyadi churna (Mean ± SEM)

Treatment	Dose (mg/kg)	RBC million/cubic mm		WBC cells / cubic mm	
		16th day	31st day	16th day	31st day
Control	--	7.90 ± 0.05	8.65 ± 0.50	46.66 ± 0.70	47.4 ± 0.61
Vehicle control	Distilled Water	7.70 ± 0.70	8.58 ± 0.01	46.74 ± 1.20	47.9 ± 0.93
Amalakyadi churna	25	8.19 ± 0.07	9.62 ± 0.82	46.72 ± 2.00	47.8 ± 0.31
Amalakyadi churna	40	8.34 ± 1.20	9.75 ± 0.90	46.66 ± 0.80	47.3 ± 0.42

Table –3 The differential leucocyte count of blood in mice after15 and 30 post-treatment days with Amalakyadi churna (Number of cells expressed in %)

Treatment	**Dose (mg/kg)**	**16th day**					**31st day**				
		Monocyte	**Neutrophils**	**Eosinophils**	**Lymphocyte**	**Baso-phils**	**Monocyte**	**Neutrophils**	**Eosinophils**	**Lymphocye**	**Baso-phils**
Control	--	2.0 ± 0.199	14.4 ± 0.576	0.6 ± 0.01	83.0 ± 0.849	0	2.6 ± 0.46	15.0 ±0.370	0.6 ± 0.002	81.8 ± 0.850	0
Vehicle control	Distilled Water	2.5 ± 0.323	16.0 ± 0.319	0.5 ± 0.024	80.0 ± 0.024	0	2.8 ± 0.087	14.9 ± 0.762	0.6 ± 0.001	80.0 ± 0.753	0
Amalakyadi churna	25	3.0 ± 0.141	16.4 ± 0.228	0.4 ± 0.001	80.0 ± 4.086	0	3.6 ± 0.303	15.2 ± 0.750	0.6 ± 0.106	80.6 ± .576	0
Amalakyadi churna	40	3.2 ± 0.909	20.8 ± 1.053	0.8 ± 0.006	75.2 ±2.378	0	3.0 ± 1.010	21.8 ± 0.944	1.0 ± 0.001	74.2 ± 1.533	0

Biochemical Studies: There were no significant changes in any liver function parameters at both the treatment groups (25 and 40mg/kg b.wt. ip for 30 days), such as in the levels of ACP (acid phosphatase), ALP (alkaline phosphatase), protein, liver and glycogen, compared to control group. Significant increase or decrease in the concentrations of these would have indicated hepatocyte damage. There was significant decrease in cholesterol content of liver was also observed. Perhaps the drug acted as hypolipidemic activity and also some beneficial and reduction effects on cardiovascular risk factors (Table - 4). It is also evidenced from the previously obtained results from *Phyllanthus emblica* (15) and *Terminalia chebula* (16).

Table –4 Biochemical changes in the liver of mice after 30 days treatment with Amalakyadi churna (Mean ± SEM)

Treatment	Dose (mg/kg)	Protein (mg/100mg)	Cholesterol (mg/100mg)	Glycogen (mg/100mg)	Alkaline Phosphatase (µM/min/ 100mg)	Acid Phosphatase (µM/min/100mg)	DNA (mg/ 100mg)	RNA (mg/ 100mg)
Control	--	4. 6 ± 0.13	0.81 ± 0.00	0.26 ± 0.025	6.48 ± 1.444	5.605 ± 0.704	0.17 ± 0.069	0.85 ± 0.03
Vehicle control	Distilled Water	4.8 ± 0.13	0.86 ± 0.00	0.258 ± 0.062	6.45 ± 0.129	5.661 ± 0.463	0.179 ± 0.801	0.77 ± 0.06
A. churna	25	4.22 ± 0.11	0.5 ± 0.0 **a**	0.288 ± 0.01	6.64 ± 1.386	5.43 ± 1.421	0.109 ± 0.039	0.78 ± 0.03
A. churna	40	4.05 ± 0.08	0.39 ± 0.0 **a**	0.248 ± 0.177	6.80 ±0.198	0.05 ± 0.98	0.085 ± 0.035	0.65 ± 0.03

Note: **a**= statistically significant (>0.05%) when compared the treated groups of mice with non-drug treated vehicle control group

Mutational Studies: It is evident from the table that, the different doses of Amalakyadi churna extract did not induce any numerical or structural aberrations in mouse femur bone marrow cells after 15 and 30 post treatment days.

Histopathology: The detailed postmortem examination has shown that the major organs like lungs, liver, stomach, heart did not show any pathological symptoms even after 30 days of treatment with Amalakyadi churna at 25 and 40mg/kg. There were a few darkly stained nuclei in the villi of intestine, medullary region of the kidney and spleen indicating mild tissue necrosis was occurred in these organs. But these organs did not show any other deformities.

In addition to the above, phytochemical screening of Amalakyadi churna extract indicated the presence of alkaloids, flavonoids, tannins, terpenoids, steroids and polyphenols (non tabulated). Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of tissue lipids. Further more reports have suggested that several plant sterols reduce serum cholesterol absorption (17). Thus it can be suggested that the synergistic interaction of polyphenols and tannin contents in the extract may impart hypolipidemic property to the herbal preparation.

Conclusion

The results of the above studies demonstrated that, the LD_{50} of Amalakyadi churna was found to be 313mg/kg b wt. intraperitoneally and it is moderatively toxic. Where as, sub-acute toxicity at 25mg/kg/day b.wt. of the drug for 30 days of treatment intraperitoneally did not induce any adverse effects. But mild toxic symptoms were observed in mice treated with 40 mg/kg b.wt. ip. This study also revealed that there was increase in the life span of mice by dose fractionation. There was no sex biased difference was found. The above parameters of toxicity studies of Amalakyadi churna revealed that this drug is a wide margin of safety at therapeutic dose level. The vital organs of mice treated with Amalakyadi churna for 30 days did not show any histopathological evidence of pathological lesions.

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