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Research Article

Acute and Sub acute Toxicity Study of Ayurvedic Formulation (AYFs) Used for Migraine Treatment

Prakash Vaidya Balendu [1*], Saraf Madhusudan [2], Chandurkar Nitin [3]

[1] *Ipca Traditional Remedies Pvt. Ltd., Mumbai, India*
[2] *Bombay College of Pharmacy, Mumbai, India*
[3] *Medical Affairs and Clinical Research, Ipca Laboratories Ltd., Mumbai, India*

ABSTRACT:
Propose: A combination of five classical ayurvedic formulations (Narikela Lavana, Sootashekhara Rasa, Sitopalad Churna, Rason Vati and Godanti Mishran) has been employed as prophylactic remedy for migraine. These ayurvedi formulations (AYFs) contain certain Bhasma and plant materials. An investigation was initiated to evaluate safety profil of these AYFs in Sprague Dawley rats and Swiss Albino mice following OECD guidelines. **Material and Method** Acute toxicity studies were done after ingestion of 5 g/kg of AYFs in a day in both the animal species. Sub acute toxicit studies were carried in five different groups in which AYFs was administrated in various doses ranging from 1.47 – 6.4 g/kg for mice and 0.7 – 7.45 g/kg for rats. The highest dose were 10 times higher that the recommended human dose Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals **Results**: The AYFs was well tolerated and no toxic manifestations were seen in any animal. Mortality observed in hig dose groups; 4% in rats and 6% in mice was not related to treatment. **Conclusion:** The AYFs was found to be safe i animals. However, chronic toxicity studies are required to know the long term safety of these AYFs.

INTRODUCTION

In the recent years complementary and alternative medicine (CAM) has upsurge globally for the treatment and prevention of many aliments which are non-communicable and chronic in nature [1]. Most surveys agree that herbal remedies are amongst the most prevalent therapies and that headache/migraine is one of the most frequent reasons for trying plant-derived medications [2]. CAM is often perceived by the public to be more helpful than conventional care for the treatment of headache [3]. However, there is always apprehension about their safety, efficacy, toxicity and reproducibility of CAM therapies.

Ayurveda the traditional system of medicine of India was initially taught and practiced in a *Guru-Shisya Parampara* has now been institutionalized. Ayurveda is largely practiced in India under the patronage of Central and Provincial Government. An Ayurvedic Treatment Protocol (AYTP) developed by the principal author was tried for migraine treatment with encouraging results [4, 5] this AYTP consist of a herbo-mineral combination of five classical ayurvedic formulations (Narikela Lavana, Sootashekhara Rasa, Sitopaladi Churna, Rason Vati and Godanti Mishran) [6] along with regulated diet and lifestyle modification. The same AYTP was used by other ayurvedic physicians with similar results[7].

Medicines of AYTP were derived from *Rasa Sashtra* (the science of Ayurvedic Pharmaceutics) and contain substances which are moderate to severely toxic in the raw form. Substances of plant, animal and mineral origin are routinely used in manufacturing of ayurvedic medicines [8]. However, the intrigue phenomenon of its manufacturing converts these into complex mineral forms which are effective and non toxic. However, improper processing/manufacturing of ayurvedic medicines may result into severe toxicity [9]. Recently, heavy metal contamination was also reported in some ayurvedic medicines sold in USA [10]. This raised concerns regarding safety of such products for human use as medicines. Hence, the present investigation was undertaken to assess the safety profile of the ayurvedic formulations (AYFs) used in the AYTP for migraine treatment in animal models using OECD guidelines.

MATERIALS AND METHODS

Animals: Six to eight weeks old male Sprague Dawley rats weighing 170 to 210 gms and female rats weighing 150 – 170 gms and Swiss Albino mice of either sex weighing between 18 - 22 gms were selected for the present study. The animals were kept in polypropylene cages with stainless lid with rice husk bedding. Individual animal was identified by specific marking and cages were identified with label pasted on cages with relevant information. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30 to 70 %. A 12:12 light: dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. The animals were acclimatized for 7 days before starting the experiment.

Ethical Clearance: This study was approved by the Institutional Ethics Committee of the Bombay College of Pharmacy, Mumbai.

**Author for Correspondence: balenduprakash@gmail.com*

Study Drugs: The recommended daily human dose of AYFs is 7.3 gm/day. The break up of which as follows: Narikela Lavana 2.0 gm; Sootshekhara Rasa 0.375 gm; Sitopiladi Churna 1.425 gm; Rason Vati 3.0 gm; Godanti Mishran 0.5 mg. The ayurvedic formulations were manufactured at Bharat Bhaishajya Shala Pvt Ltd, Dehradun, India. Based on the doses of these individual formulations the equivalent animal dose were calculated for the mixture of AYFs in the same proportion these are used in human being.

Dose administration: AYFs were triturated to get fine powder and were mixed uniformly in the required proportion. The suspension for low, medium and high doses were prepared in 0.5% Carboxy Methyl Cellulose in distilled water and administered to animals orally with the help of gastric catheter.

Acute Toxicity study: Six female rats and mice were orally administered 5 g/kg suspension of the mixture of AYFs in three divided doses, at an interval of 30 minutes in a day. The equivalent human dose per day of these AYFs in rat and mice was calculated to be 0.7 g/kg and 1.47 g/kg respectively. The dose administered in rat and mice was approximately 7 times and 3.4 times higher than the normal human dose respectively. The animals were observed for first 4 hours of treatment to next 14 days. The evaluated parameters were mortality, signs and symptoms of toxicity, body weight, food consumption and necropsy observations.

Sub Acute Toxicity study: The animals were divided into 5 groups. There were 10 animals (5 males and 5 females) in each group. The group I served as vehicle control. Group II, III and IV received low, medium and high dose of medicine respectively for 28 days. All the animals of group I – IV were sacrificed on 29th day. However, group V received high dose medicine for 28 days, and then they were further observed for next 2 weeks and sacrificed on 43rd day. The dose was calculated taking into account the difference in surface area: body weight ratios between species. The details of dosing are given in the box below:

Study parameters: Toxic manifestations like alteration in water or food intake, weight loss, respiration pattern, mobility, response to handling, salivation, piloerection, bizarre behavior were studied on day 0 and every week thereafter. Audio-visual reflexes and grip strength determination was performed in all animals at 4th week of study. Opthalmological observation was done on day 1 and day 28. Parameters like pupil size, redness of eye and lacrimation were checked and scored. Blood samples were collected from all animals after terminal sacrifice. Hematological and biochemical parameters viz. hemoglobin (Hb), red blood corpuscles (RBC), white blood corpuscles (WBC) and platelets (Plt), reticulocyte (Reti), packed cell volume (PCV), prothrombin time (PT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) , Alanine transaminase (SGPT), Aspartate transaminase (SGOT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), random blood sugar (RBS), sodium (Na^+), potassium (K^+), calcium (Ca^{++}), phosphorus (P), chloride (Cl) and cholesterol (CHO). Biochemical parameters were studied using commercially available kits, Span autochem 2011 autoanalyzer and flame photometry.

Necropsy: Body orifices and organs of all animals were carefully observed after dissection for morphological and pathological changes. Wet weight of liver, kidney, spleen, brain, heart, adrenals and gonads (testes/ovaries) were recorded for all animals.

Histopathology: Tissue samples from various organs of all animals were preserved in 10% formalin saline and were studied for pathological changes.

Statistical analysis: The data was analyzed by using SPSS software (version 12.0, SPSS, Chicago, IL, USA). The results are presented as mean ± s.d. and statistical significance between the groups was analyzed by means of an analysis of variance (ANOVA).

RESULTS

Acute Toxicity study: There were no signs of any toxicity in animals of both the species after the administration of the test dose of 5 g/kg suspension of the mixture of AYFs. All the animals showed similar food intake, body weight gain and clinical signs as that of the control group. No morbidity or mortality was observed in the treated animals. The necropsy studies did not detect any abnormality.

Sub Acute Toxicity study: The animals from both the species showed normal body weight gain throughout the dosing period. No significant change in weight was noted in any of the groups. There was no difference in the food intake noted in rats. However, significant reduction in food consumption in mice was noted from second week of study in the medium dose, high dose and satellite treatment group in comparison to control group.

Group	Dose	Rat	Mice	Treatment (days)	Day of sacrifice
		(g/kg body weight/ day)			
I	Control	--	--	28	29
II	Low	0.7	1.47	28	29
III	Medium	2.23	3.04	28	29
IV	High	7.45	6.48	28	29
V	High	7.45	6.48	28	43

One rat each from group IV and V died on 4th and 5th week of study. Three mice died during the study period. Two mice from group IV and V died in the 1st week of study. One mouse died at the 4th week of treatment from group V.

Table 1: Comparison of Group Mean Hematological Investigations in Rats

Group	***Control***	***Low dose***	***Medium dose***	***High Dose***	Satellite high dose
Hemoglobin (gm/dl)	12.1± 1.0	12.4± 0.98	12.5 ± 0.96	12.9 ± 0.87	13.0 ±0.74
RBC (x 10^6/cmm)	7.27 ±0.52	7.09 ± 0.58	6.83 ± 0.64	7.5 ±0.57	7.56 ± 0.53
WBC (x 10^3/cmm)	9.360 ± 2.249	8.950 ±2.089	7.970 ±1.567	6.690 ±2.060	8.090 ±1.491
Platelets (x 10^5/cmm)	9.472±6.8171	9.788 ± 4.738	10.242±5.859	10.120± 1.206	9.094±5.607
Reticulocytes (%)	2.46 ±0.49	2.79 ± 0.78	2.56 ± 0.55	2.24 ± 0.69	1.36 ± 0.56*
PCV (μ m^3)	37.7 ± 3.84	37.6 ± 4.03	38.0 ± 3.33	39.8 ± 2.88	41.2 ± 3.47
MCV (μ m^3)	52.5 ± 3.6	52.9 ±3.33	55.6 ± 2.75	52.6 ± 4.01	55.3 ± 2.46
MCHC (gm/dl)	32.5 ± 2.03	33.0 ± 0.95	33.1 ± 0.96	32.4 ± 1.24	31.6 ± 1.03
MCH **(pg)**	16.7 ± 0.89	17.5 ± 0.97	18.4 ± 1.11*	17.2 ± 1.38	17.5 ± 0.76

Values are mean of 10 animals ± S.D.
**** Significant at 95 % level of confidence (p<0.05) vs. control group.***

Table 2: Comparison of Group Mean of Hematological Investigations in Mice

Group	***Control***	***Low dose***	***Medium dose***	***High Dose***	Satellite high dose
Hb (gm/dl)	13.87± 0.8	13.56± 0.6	14.56± 0.5	12.97± 3.3	14.18± 0.6
RBC (x 10^6/)	9.2 ± 0.8	9.1± 0.5	9.3± 0.6	8.23±1.3	7.9± 0.6
WBC (x 10^3/cmm)	8.700 ±2740	7.580± 1452	8.210± 1733	8.240± 3714	9.550± 1676
Platelets (x 105/cmm)	7.970 ±4478	8.389± 7440	8.934 ±9531	7.992±19330	8.547 ± 9856
Reticulocytes (%)	0.9±0.35	0.9± 0.31	1.0 ± 0.22	1.0 ± 0.22	1.0 ± 0.29
PCV (μ m^3)	45.4 ± 4.5	44.1 ± 3.9	45.8 ± 2.9	39.9 ± 9.8	42.1 ± 2.7
MCV (μ m^3)	50.15 ± 5.1	48.85 ± 3.6	49.0 ± 1.5	49.53 ± 6.3	52.8 ± 6.7
MCHC (gm/dl)	30.4± 1.9	30.7 ± 2.0	31.91± 1.5	32.18 ± 0.4	32.7 ± 0.4
MCH **(pg)**	15.2 ± 1.2	15.1 ± 0.6	15.6 ± 0.7	15.7 ± 1.9	17.6 ± 0.8*

Values are mean of 10 animals ± S.D.
**** Significant at 95 % level of confidence (p<0.05) vs. control group.***

Animals from all treated groups in both the species showed a normal hematological profile except for the MCH value of the medium dose group in rats (Table 1) and satellite group in mice (Table 2) was significantly higher from that of the control group. The reticulocyte count in the satellite group was also significantly higher than the control group in rats.

Animals from all treated groups in both the species showed a normal biochemical profile including electrolyte levels (Table 3, 4, 5 & 6) except for the albumin levels in the satellite group in mice which was significantly higher from that of the control group (Table 5).

Treated animals from both the species showed organ weights as well as organ to body weight ratio comparable to control group.

Table 3: Comparison of Group Mean of Biochemical Investigations in Rats

Group	Control	Low dose	Medium dose	High Dose	**Satellite high dose**
AST (IU/L)	143.5 ± 22.4	136.5 ± 8.7	144.6 ± 13.4	160.2 ± 18.8	127.6 ± 5.2
ALT (IU/L)	49.3 ±10.1	50.3 ±11.3	39.0 ± 6.9	43.0 ± 10.9	56.4 ± 11.8
ALP (IU/L)	388.3 ± 90.1	458.5 ± 84.6	333.4 ± 32.7	364.4 ± 87.1	394.1 ± 65.4
Protein (g/dl)	5.87 ± 0.35	5.86 ± 0.47	6.03 ± 0.82	6.18 ± 0.36	6.59 ± 0.36
Albumin (g/dl)	4.07 ± 0.37	4.7 ± 0.88	4.59 ± 0.83	4.26 ± 1.04	3.7 ± 0.44
BUN (mg/dl)	39.9 ± 10.2	37.0 ± 9.5	35.7 ±12.6	38.0 ± 9.01	28.9 ±8.02
CRE (mg/dl)	0.33 ± 0.27	0.71 ± 0.31	0.33 ± 0.41	0.43 ± 0.43	0.67 ± 0.72
RBS (mg/dl)	117.8 ± 30.5	129.1 ± 2.55	113.8 ± 16.5	121.2 ± 28.2	119.2 ± 25.3
CHO (mg/dl)	137.7 ± 4.1	145.6 ± 21.5	153.5 ± 46.7	152.6 ± 21.9	141.9 ± 25.7
BIL (mg/dl)	0.53 ± 0.14	0.5 ± 0.12	0.56 ± 0.17	0.45 ± 0.1	0.5 ± 0.13

Values are mean of 10 animals ± S.D. No significant difference was observed in any parameter.

Animals from high dose treatment group and satellite group of both the species showed decreased motor activity (reduced alertness, reduced exploratory behavior). The effect lasted for approximately two hours post dose administration. However, the animals responded comparably to control group in the functional test on retard. All the other treatment animals were found to be free of any intoxicating sign.

Treated animals of both the species showed normal ophthalmologic and audio-visual reflex at the end of study period. Gross pathological examination of all animals did not reveal any abnormality attributable to the treatment in both the species. No significant histopathological changes were noted in different organs that were examined.

DISCUSSION

In the present investigation we tried to assess the safety profile of AYFs. No Adverse Effect Level (NOAEL) could be established for all the dose range tested in both the animal models. The higher dose range tested was well above and at an adequate safety distance of the recommended dose in humans. Though the animals treated with high dose of medicines showed reduced alertness and decreased motor activities, they responded comparably to control group in the functional test on rotarod. It may be assumed that the AYFs at high dose may have mild CNS depressant activity. Mortality observed was not found to be related to treatment.

Table 4: Comparison of Group Mean of Electrolyte Levels in Rats

Group	*Control*	*Low dose*	*Medium dose*	*High Dose*	**Satellite high dose**
Na	132.4 ± 41.7	147.0 ± 4.98	147.4 ± 5.39	147.7 ± 3.97	145.9 ± 3.9
Ca	8.5 ± 2.54	9.58 ± 1.16	9.43 ± 1.22	9.25 ± 0.82	8.98 ± 1.6
P	5.5 ± 0.77	5.7 ± 0.5	6.1 ± 1.22	5.9 ± 1.32	6.8 ± 1.84
K	4.58 ± 0.36	4.83 ± 0.47	4.71 ± 0.47	4.74 ± 0.39	4.52 ± 0.43
Cl	105.8 ± 2.61	107.8 ± 4.15	107.1 ± 5.56	107.1 ± 5.56	107.3 ± 3.62

Values are mean of 10 animals ± S.D. No significant difference was observed in any parameter.

The present investigation indicated that the ayurvedic formulations did not produce any adverse toxicity in both

Table 5: Comparison of Group Mean Biochemical Investigations in Mice

Group	*Control*	*Low dose*	*Medium dose*	*High Dose*	**Satellite high dose**
AST (IU/L)	95.5 ± 10.7	103.6 ± 18.8	97.2 ±16.86	160.6 ± 17.1	93.1 ±15.36
ALT (IU/L)	44.4 ± 10.16	54.0 ± 16.5	56.5 ± 13.9	52.0 ± 23.7	53.0 ±13.6
ALP (IU/L)	286.5 ± 47.4	292.5 ± 22.5	332.9 ± 42.9	295.4 ± 46.5	283.8 ± 28.8
Protein (g/dl)	7.01± 0.7	7.1 ± 0.6	6.94 ± 0.7	7.08 ±0.7	6.97 ± 0.3
Albumin (g/dl)	2.83 ± 0.4	2.75 ± 0.3	2.85 ± 0.4	3.06 ± 0.2	3.47 ± 0.2*
BUN (mg/dl)	54.0 ± 20.1	53.3 ± 11.4	65.1 ± 21.2	82.1 ± 41.6	77.1 ± 13.0
CRE (mg/dl)	1.5 ± 0.06	0.6 ± 0.45	1.2 ± 0.67	0.81 ± 0.55	0.7 ± 0.41
RBS (mg/dl)	124.2 ± 41.6	152.6 ±36.0	121.8 ± 25.4	122.6 ± 50.7	148 ± 27.2
CHO (mg/dl)	124.5 ± 17.2	126.0 ± 25.1	123.5 ± 22.0	131.6 ± 22.2	120.2 ± 15.1
BIL (mg/dl)	0.44 ± 0.1	0.92 ± 1.43	0.48 ± 0.12	0.46 ± 0.12	0.51 ± 0.16

Values are mean of 10 animals ± S.D.
**** Significant at 95 % level of confidence (p<0.05) vs. control group.***

the animal models studied. There was no pathological evidence of toxicity in kidney, liver, spleen, heart and

brain. Mortality observed in the treatment groups was not found to be dose related. The animals that died did not show any signs of morbidity and necropsy. The histopathology findings did not indicated any toxic changes in these animals.

The presence of metals and minerals in food / ayurvedic medicines is a matter of great concern for human health [11]. The AYFs used in the present study is a combination of 5 ayurvedic formulations derived from *Rasa Aushadi* and contained herbs, metals and minerals. However, classic Rasa text claims the intrigue processing of metals and final composition of its formulations are safe

for human consumption and therapeutically effective. The preliminary observational research indicates that AYFs are significantly effective in the prevention of migraine and need not produce any noticeably side effect among migraineurs [4, 5].

Normally, heavy metals produce nephrotoxicity and blood disorders. However, these heavy metals after subjecting to specific and proper processing are transformed into Bhasma[12] and used in treatment of various ailments and are considered as nontoxic in prescribed dose. Bhasma along with appropriate herbs are used for the treatment of critical ailments. The procedures for preparing these medicines are stringent, time-consuming and complicated [13]. Metals are triturated and burnt several times [14] with herbs juices / decoction thereby converting them into non toxic form and suitable for clinical usage. It is estimated that 35% to 40% of the approximately 6000 medicines in the ayurvedic formulary intentionally contain at least one metal. Metal-containing herbal medicine products are purportedly "detoxified" through multiple heating/cooling cycles and by the addition of specific herbs [15].

Ayurvedic is largely practiced using ancient protocols and parameters. Though there is a need of scientific scrutiny of its principles of treatment, very few attempts have been made for its scientific and systemic validation. The present study indicates that the AYFs that are clinical used are also safe in animals in dose that was 4 - 10 times higher the human equivalent dose. However, further studies are required to know the long term chronic toxicity of these AYFs.

Table 6: Comparison of Group Mean of Electrolyte Levels in Mice

Group	*Control*	*Low dose*	*Medium dose*	*High Dose*	**Satellite high dose**
Na	142.8 ± 5.3	142.3 ± 3.4	142.4 ± 4.3	142.5 ± 4.05	144.9 ± 5.04
Ca	8.27 ± 2.6	9.09 ± 1.18	9.4 ± 1.74	9.72 ± 0.75	9.54 ± 0.85
p	7.63 ± 1.43	8.19 ± 1.3	7.32 ± 0.69	7.47 ± 1.3	8.23 ± 0.97
K	4.64 ± 1.33	4.83 ± 0.7	4.49 ± 0.84	4.49 ± 0.66	4.23 ± 0.94
Cl	105.8 ± 2.61	107.2 ± 4.23	107.4 ± 4.16	108.3 ± 5.85	107.2 ± 4.21

Values are mean of 10 animals ± S.D. No significant difference was observed in any parameter.

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