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Evaluation of Acute and Long-term Toxicity of Siddha Formulation *Annabethi Chendhuram*

Research Article

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Abstract

Introduction: *Annabethi Chendhuram* is extensively used for the treatment of anaemia by Siddha practitioners. But concerns about the safety of drugs are raised in the current era due to a lack of scientific validation. Aim: The present study was conducted to determine the acute and long-term toxicity of *Annabethi chendhuram.* Methods: Acute and long-term toxicity of *Annabethi chendhuram* were evaluated as per WHO guidelines in Wistar albino rats. In the acute study, 250mg/kg.b.wt of test drug was given for animals then observed for any toxic signs and mortality up to 14 days. In a long-term toxicity study, a test drug was administered at the dose of 25, 125 and 250mg/kg/b.wt/p.o/day for animals up to 90 days and observed for mortality and morbidity. All animals were sacrificed at the end of the study. Haematology, biochemical parameters and histopathological examination were analyzed. Result: In the acute toxicity study, no toxic signs or death were observed in all animals. In the long-term toxicity study, no significant differences were observed in body weight changes, food intake, water intake, haematology, biochemical parameters and histopathology of both control and test groups. Conclusion: The study result concludes that the long-term oral administration of 250 mg/kg.b.wt of *Annabethi chendhuram* did not cause any toxic effect in rats. So, the drug is safe for human consumption.

Key Words: *Annabethi chendhuram,* Anaemia, Acute toxicity study, Long term toxicity study, WHO guidelines.

Introduction

Anaemia is a global public health crisis, affecting 1.62 billion of the world population.(1) It is the condition in which red blood cell and haemoglobin concentration in the blood are lower than normal and are inadequate to meet individual physiological needs. (2) According to the World Health Organization, around 32.4 million pregnant women suffer from anaemia worldwide.

Anaemia in pregnancy is a serious health problem that increases the risk of maternal morbidity and mortality.(3) It also affects fetal growth and development which in turn raises the percentage of infant mortality.(4) About 50% of maternal deaths are associated with anaemia.(1) Among them, Iron deficiency anaemia is the most common type, since iron requirements increase three times more in pregnancy than in menstruating women.(5) Iron supplementation to pregnant women helps to improve perinatal outcomes.(6) But oral iron supplements produce adverse effects such as heartburns, nausea, upper gastrointestinal disturbances, constipation, diarrhoea, and tooth stains.(7)

The government of Tamil Nadu launched the scheme of Amma Magaperu Sanjeevi kit in 2016 for the welfare of pregnant women and infants. The kit contains 11 Siddha medicines which were given to pregnant women to reduce nausea, vomiting, balance iron deficiency, reduce false pain and improve immunity in infants.(8) *Annabethi chendhuram* is one among the eleven Siddha medicines of the Amma Magaperu Sanjeevi kit which helps to reduce the complications of iron deficiency anaemia in pregnant women as well as infants.

Annabethi chendhuram (ABC) is a Siddha formulation consisting of *Annabethi* and lemon juice. It is indicated to treat anaemia, fever and dysentery in Siddha literature.(9) It is commonly used for the treatment of Anaemia (*Paandu*) by Siddha practitioners for a long period. But till now there is no scientific background for the safety of *Annabethi chendhuram*. Regulatory guidelines strongly suggested that the safety profiling of drugs is essential before their use in clinical research.(10) Though Siddha drugs were used over a long period, safety profiling of drugs is done in this study as reverse pharmacology for the recognition of the scientific community. So, the present study was conducted to evaluate the acute and long-term toxicity of *Annabethi chendhuram* on Wistar albino rats as per World Health Organization (WHO) Guidelines.

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Materials And Methods

Preparation of *Annabethi chendhuram*

35 grams of purified *Annabethi* was placed in the *kalvam* and ground by adding lemon juice. Then the ground substance was made into a cake and dried. After that, it was put in a flask, calcined (*Pudam* process) thrice and stored.(9)

Toxicity Profiling of *Annabethi chendhuram*

The toxicity study was done after obtaining approval from Institutional Animal Ethics Committee (IAEC No: NIS/IAEC-II/12/2016 dated 29.9.2016) at the animal house, National Institute of Siddha, Chennai as per WHO Guidelines.(11)

Animal selection

Healthy adult Wistar albino rats of both sexes, weighing 140-160 g were obtained from TANUVAS, Madhavaram, Chennai were used for the study. The animals were kept in polypropylene cages. A 12-hour light / dark cycle was maintained. Room temperature was maintained between 22±3°C and relative humidity 30–70%. They were provided with Rodent pelleted food and RO purified water ad libitum. All the animals were acclimatized in the laboratory for 7 days before the beginning of the study.

Dose fixation

As per Siddha's text, the therapeutic dose of *Annabethi chendhuram* is 65-130 mg. The suitable dose for rats was calculated as per Paget and Branes (12) and was found to be 25, 125 and 250 mg/kg body weight of rat for low dose, mid dose and high dose groups respectively.

Acute Toxicity Study

The animals were divided into 2 groups with 10 animals (5Male + 5Female) in each group. Group, I set as control and administered honey. Group II set as test group treated with 250 mg/kg b. wt of *Annabethi chendhuram*. The animals were fasted for 12hrs and weighed before dosing. Honey and a single dose of the test drug (250mg/kg/body weight) were administered to Group I and Group II animals respectively by oral gavage using an intubation cannula. The food was withheld for another 4 hours after administration of the drug. The animals were observed individually after dosing for the first 30 mins, then periodically during the first 24hour, with special attention given during the first 4hours, and thereafter twice daily for 14 days. Bodyweight was noted once a week. Feed intake and water intake were calculated daily. Observations like mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, gripping, grooming, exophthalmos, diarrhoea, salivation, lacrimation, posture, dyspnoea and coma were examined visually for 14 days. At the end of the 14th day all the animals were sacrificed by using the injection of thiopental sodium. All animals were subjected to gross necropsy and observed for pathological changes.

Long-term Toxicity Study

Wistar albino rats of both sexes were divided into four groups. Each group consists of 20 animals (10 Male +10 Females). The first group treated as control, second, third and fourth group were set as Low dose, Mid dose and High dose respectively. The control animals were administered, honey. Low dose, Mid dose and High dose were treated with 25, 125 and 250mg/kg/b. wt of *Annabethi chendhuram* orally for 90 days. Animals were monitored for behavioural parameters daily after drug administration. The bodyweight of the animal was monitored at weekly intervals. The food and water intake were calculated daily. On the 91st day, all animals were weighed and sacrificed by using the injection of thiopental sodium. Blood was collected from the abdominal aorta of anaesthetized animals and stored for haematological and biochemical analysis. Gross necropsy was observed and organs were preserved in formalin for histopathological evaluation.(11)

Hematological analysis

Blood samples of control and experimental rats were analyzed for haemoglobin, total red blood corpuscles, White blood corpuscles count, platelet, mean corpuscular volume, mean corpuscular haemoglobin and differential count by using an auto analyzer.

Biochemical analysis

Biochemical parameters such as Lipid profile test (Total cholesterol, High-density lipoprotein, Low-density lipoprotein, Very low-density lipoprotein, Triglycerides), Liver function test (SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase), Total bilirubin), and Renal function test (creatinine and urea) were analyzed.

Histopathological analysis

Control and high dose group animals were initially subjected to histopathological investigation. Various organs such as brain, heart, lung, liver, kidney, spleen, stomach, uterus, testes, ovary were collected and preserved in 10% buffered neutral formalin, sliced as 5 or 6 µm sections, stained with hematoxylin and Eosin stains and then examined under a microscope for histopathological changes.

Statistical analysis

Statistical analysis of findings such as body weight changes, food intake, water intake, haematology and biochemical parameters were carried out by one-way ANOVA using a computer software program *Graph Pad Instant-3*. Dunnet's test was used to compare control and test drug-treated groups. P-value < 0.05 was considered the level of significance.

Results

Acute toxicity study

In the Acute toxicity study, there was no treatment-related death or signs of toxicity observed in Wistar albino rats throughout the study period at the dosage of 250mg/kg b.wt (Table.1). Further, no gross pathological changes had been seen in the internal organs of both controls and treated groups.

Table.1. Effect of *Annabethi chendhuram* on Behaviour signs parameters of Wistar albino rats

S. No	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	Test 250 mg/ kg /b.wt	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch response 7.Decreased Motor Activity 8.Tremors 9.Convulsion 10.Muscle spasm 11.Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exopthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20.Mortality

(+) Presence of activity; (-) Absence of activity

Long-term Toxicity Study

In the Long-term toxicity study, no toxic signs were observed in behavioural parameters of all the control and test group treated animals. The body weight was significantly increased in the test group when compared to the control group. Food intake and water intake significantly increased in the high dose group when compared to the control group. Haematological parameters revealed a statistically significant decrease in MCV value of mid-dose group animals when compared to the control group but it was within the physiological limit (Table.2). In biochemical parameters, a statistically significant increase in Triglyceride level was noted in the low dose group when compared to the control group but it was within the physiological limit (Table.3). The other haematological and biochemical parameters such as lipid profile, liver function test and renal function test were normal in test groups when compared with the control group. The histopathological analysis of organs such as brain, heart, lung, kidney, spleen, liver, stomach and reproductive organs revealed no abnormalities in all high dose groups when compared with control group animals.

Table.2. Effect of *Annabethi Chendhuram* on Haematological parameters of Wistar albino rats

Parameter	Control	Low dose	Mid dose	High dose
RBC ($\times 10^6 \mu l$)	6.86±1.12	6.41±2.05	6.96±1.05	6.83±1.13
WBC($\times 10^3 \mu l$)	8.86±2.38	9.06±1.76	9.9±2.40	8.5±2.56
PLT($\times 10^3 \mu l$)	637±169.52	698±207.18	722.5±163.5	714.6±177.01
HB(g/dl)	13.24±1.30	12.4±1.59	13.93±1.34	13.33±1.064
Neutrophils	1.74±0.55	1.67±0.65	1.71±0.66	1.75±0.55
Eosinophil's (%)	1.33±0.18	1.46±0.3	1.47±0.66	1.5±0.25
Lymphocyte(%)	76.84±9.11	79±9.24	77.02±11.55	78.45±14.47
Monocyte(%)	3.18±0.65	2.58±0.98	3.36±1.26	2.6±0.84
Basophils(%)	0.3±0.48	0.1±0.31	0.2±0.42	0.1±0.31
MCH(pg)	18.44±2.08	19.79±3.06	19.39±2.68	17.79±2.53
MCV (fl)	61.46±5.71	62.8±6.23	60.23±6.64**	61.51±5.25

Values were expressed as mean± S.D. for N=10 rats in each group; * - P<0.05; ** - P<0.01

RBC- Red blood cell; WBC- White blood cell; PLT- Platelet count; HB- Haemoglobin; MCV- Mean corpuscular volume; MCH-Mean corpuscular haemoglobin

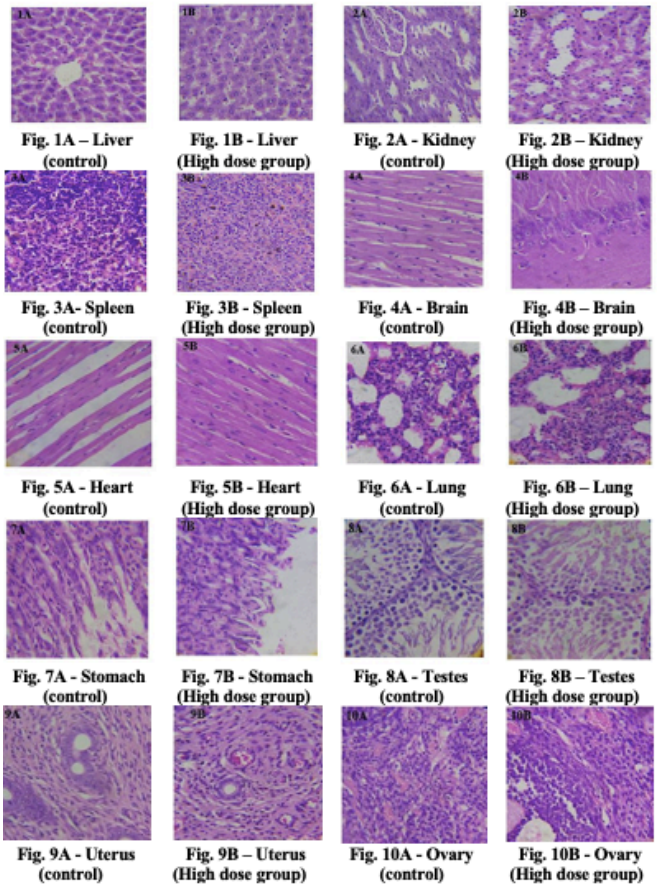
Table.3. Effect of *Annabethi Chendhuram* on Biochemical parameters of Wistar albino rats

Parameter	Control	Low dose	Mid dose	High dose
Total cholesterol (mg/dl)	155.47±17.87	148.15±15.08	148.8±19	152.2±18.23
HDL (mg/dl)	60±8.13	59.2±8.44	59.6±7.96	62.5±8.22
LDL (mg/dl)	72.7±13.78	63±17.72	70.7±9.77	72.4±15.37
VLDL (mg/dl)	18.32±3.69	16.73±4.19	16.68±4.71	17.93±3.36
Triglycerides(mg/dl)	30±10.36	45.9±10.54**	34.4±7.50	13.6±12.48**
BUN(mg/dl)	14.1±2.46	15.87±2.67	16.7±4.01	15.82±3.88
Creatinine(mg/dl)	0.77±0.24	0.77±0.26	0.71±0.19	0.77±0.18
Total Bilirubin(mg/dl)	0.32±0.13	0.33±0.13	0.39±0.16	0.35±0.08
SGOT(U/L)	123.7±26.61	113.8±14.45	131±29.93	122.8±31.22
SGPT(U/L)	34.4±6.29	32.1±8.73	27±9.28	35.3±5.98

Values were expressed as mean± S.D. for N=10 rats in each group; * - P<0.05; ** - P<0.01

HDL-High density lipoprotein; LDL-Low density lipoprotein; VLDL-Very low density lipoprotein; BUN-Blood urea nitrogen; SGOT-Serum glutamic oxaloacetic transaminase; SGPT- Serum glutamic pyruvic transaminase

Figure.1. Histopathology of control and ABC treated rats in the long term toxicity study



Discussion

Annabethi chendhuram has been used for the management of anaemia by Siddha practitioners over a long period. But the safety of ABC has not been scientifically validated till now. So, in this study acute and long-term toxicity of ABC was evaluated in Wistar albino rats as per WHO guidelines.

In the Acute toxicity study, there were no abnormal signs reported at the dose level of 250 mg/kg/ b.wt in Wistar Albino Rats. No mortality and gross pathological changes were noted in the internal organs of both control and ABC treated groups.

In the Long-term Toxicity Study, no toxic signs were observed in the behavioural parameters of all the control and ABC treated groups. The body weight, food intake and water intake were significantly increased in the test group when compared to the control group. The haematological parameters serve as a sensitive index for pathological conditions both in humans and animals.(13) Results of haematological parameters were normal in test groups when compared to the control group except MCV (Mean corpuscular volume) which was significantly reduced in mid-dose group animals, but the value was within the physiological limit. So, the test drug ABC did not cause any hematopoietic toxicity in rats.

Biochemical parameters have a major role in determining the toxicity of drugs because these parameters are represented by signs of disease.(14) In the present study, regarding triglyceride levels in the Lipid profile, Low dose group animals show a significant increase than that of control group animals. Moreover, the increased value also lies within the physiological limits. These changes were not observed in the mid and high-dose group animals and this may be due to the influence of its food intake.

Hepatocellular damage may lead to a reduction in serum concentrations of albumin, total protein and bilirubin. SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamic pyruvic transaminase) are generally used as markers for liver damage.(15) Urea and creatinine are sensitive indicators of renal toxicity.(16) In this study, Renal function tests and liver function tests were normal in ABC treated group as well as in a control group. So, the test drug did not cause any damage to the liver and kidney cells.

The evaluation of histopathological changes in organs remains a basis in the safety of medicines. In this study, histopathology of organs such as brain, heart, lung, kidney, spleen, liver, stomach and reproductive organs revealed no cellular changes in high dose and control group animals. From the present study, it is revealed that the long-term administration of ABC did not cause any toxicity to rats and the drug is safe. A clinical study by R. Sivaraj et al also showed no adverse effect in 20 ABC treated patients except constipation. (17) Constipation may be due to the presence of annabethi in ABC, which may be relieved by administering *Annabethi chendhuram* with honey which has laxative properties as mentioned in Siddha literatures.(9)

Conclusion

The toxicity study result of ABC did not show any toxic effects on rats at the dose level of 250 mg/Kg b.wt. So, it is concluded that the therapeutic dose of *Annabethi chendhuram* (65 to 130 mg) is safe for human consumption. Further clinical studies will be needed on ABC to prove its efficacy and safety in humans.

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