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Evaluation of Acute and Subacute toxicity of Siddha Polyherbal formulation *Chitramutti Nei*

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Abstract

Chitramutti Nei (CN) is the poly herbal Siddha formulation has been used in treatment of anaemia, jaundice, fever etc., To evaluate its safety, acute and sub acute oral toxicity studies were performed following OECD test guidelines 423 and 407, respectively. In acute oral toxicity study, *CN* was administered at 2000mg/kg orally in wistar albino rat models . The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality. In 28 day repeated dose toxicity study, CN was administered at 200 and 400 mg/kg body weight daily, for 28 days. In acute toxicity study, no treatment related death or toxic signs were observed. It revealed that the LD_{50} cut-off value of CN is greater than 2000 mg/kg body weight. The 28 day repeated dose study did not show evidence of any treatment related changes in all observations up to the high dose level. There is no significant differences in organ weight, haematological and biochemical parameters of treated animals when compared with the control. Histopathological examination revealed no abnormalities. It revealed that the NOAEL > 400mg/kg/day in Wistar albino rats.

Keywords: Chitramutti Nei, Anaemia, acute oral toxicity, Sub acute toxicity.

Introduction

Anaemia is a condition characterized by reduced circulating hemoglobin in the body and it often act as a major disease or as a secondary condition associated with many other diseases. Iron deficiency is the major contributor to anaemia globally. In our country Nutritional Iron Deficiency is the most common cause of anaemia. 'Iron Deficiency Anaemia'(IDA) is a very common disease prevalent in the society. World Health Organization (WHO) estimates that prevalence of anaemia is 14% in developed countries, 51% in developing countries, and 65 to 75% in India.[1] Iron deficiency is defined as a condition in which there is depletion of moblizable iron stores in the body and associated with signs of insufficient supply of iron to tissues. The more severe stages of iron deficiency are associated with anaemia. When individual hemoglobin levels are below two standard deviations (-2SD) of the distribution mean for haemoglobin in an otherwise normal population of the same gender and age who are living at the same altitude, IDA is considered to be present.[2] Long term oral iron therapy is commonly used as first line therapy are associated with a high incidence of gastrointestinal side effects such as nausea, vomiting, diarrhoea or constipation. Because of their adverse effects, a safe, effective, cheap, and easily available drug is needed. Many drugs are available in siddha system of medicine which have remarkable effects in treating anaemia. One such medicine is *Chitramutti Nei* indicated for anaemia mentioned in Siddha classical literature. It is also

indicated for fever, jaundice and dropsy. With the aim of that, this Polyherbal preparation may be effective to manage childhood IDA without any synergistic effects. Hence the present study was carried out to evaluate the acute and sub-acute toxicity of the *Siddha* polyherbal formulation *Chitramutti Nei* in experimental rodents. Through this study the safety of this poly herbal formulation, was investigated to assess its safety and tolerability profile in long-term treatment.

Materials and Methods

2.1. Collection of raw drug and plant materials

The plant material of *Chitramutti (root) – Sida cordifolia, Karimanjal - Curcuma longa, Kadukkai - Terminalia chebula, Thendrikkai –Terminalia belirica, Nelivatral - Phyllanthus emblica, Nilavembu- Andrographis paniculata, Illupai ver pattai* (root bark) *- Madhuca longifolia , Cow's milk, Ghee* were procured from a well reputed indigenous drug shop. Fresh specimen of *Chitramutti root, Illupai ver pattai* were collected from Siddha medicinal plant garden, Mettur, Salem, Tamil Nadu. The drugs were authenticated by the concerned Pharmacognosist, SCRI, Chennai.

2.2. Purification

The collected raw drugs and fresh specimen of *Chitramutti (root), Illupai ver* pattai (root bark) were dried in sunlight and purified as per the methods defined in Siddha literature for further preparation.

2.3. Preparation of *Chitramutti Nei*[3]

The dried material of above mentioned drugs were taken in equal quantity of 280 gms and made into coarse powder. The coarse drugs were put in a mud pot. 5200 ml of water has been added and content has been boiled till the content become half. 5 lit of cow's milk and 1.5lit of ghee has been added to the above decoction and boiled the content till it reaches ghee like consistency. The entire composition has been filtered and cooled. Then the drug was stored in a clean and dry air tight container.

Experimental animals husbandary

Healthy adult Wistar albino rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 $\pm$ 2^0 C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India. **IAEC** SU/CLATR/IAEC/ VII/042/2016

Acute toxicity study

Acute toxicity study of the study drug *Chitramutti Nei* was carried out as per OECD guideline (Organization for Economic Co-operation and Development) Guideline-423.[4] The animals were fasted overnight with free access to water. The study was conducted with single oral dose administration of *Chitramutti Nei*.

Animal Grouping

One group consist of 6 female rats were used for this study. The dose utilized for evaluation of acute toxicity study is about 2000 mg/kg higher than that of the therapeutic dose.

Dose Equivalent = 1ml is equivalent to 1.02085 gm

GROUP I : Animals received Test drug 2000 mg/kg (p.o)

The animals were fasted overnight (12- 16 hrs) with free access to water. The study was conducted with single oral administration of study drug *Chitramutti Nei* 2000mg/kg equivalent to 0.4ml (p.o). The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality. Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S,C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention. Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.

Sub-acute toxicity study

Sub-acute toxicity study was carried out as per OECD guidelines Guideline-407 [5].

Animal Grouping

Animals were divided into three groups of 06 animals each consist of 3 male and 3 female rats.

GROUP I : Animals received saline 5 ml/kg b.w (p.o)

GROUP II : Animals received low dose of test drug 200 mg/kg (p.o)

GROUP III : Animals received high dose of test drug 400 mg/kg (p.o)

The animals were randomly divided into control group and drug treated groups for two different doses viz. low dose (200 mg/kg b.w) equivalent to 0.2 ml and high dose (400 mg/kg b.w) equivalent to 0.4 ml,p.o per rat.

The animals were administrated with the study drug once daily for 28 days. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *Chitramutti Nei* 200 mg/kg b.w (p.o) and group III received high dose of *Chitramutti Nei* 400 mg/kg b.w (p.o).

The rats were weighed periodically and observed for signs of toxicity pertain to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra actate) for Hematological analysis and for serum generation for biochemical analysis.

The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.

Hematological analysis

Blood samples were analyzed using established procedures and automated Bayer Hematology analyzer. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

Biochemical analysis [6]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) , Very low density Lipoprotein (VLDL) , Triglycerides (TGL), Total Cholesterol , Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.

Histopathological evaluation [7]

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

Statistical analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error .A statistical comparison was carried out using the Dunnet's test for the control and treatment group.

Results

Acute toxicity study

There were no mortality and toxicity signs observed in both control and CN treated groups throughout the study period. No significant difference in toxicity signs, weight gain and no gross pathological changes in treated groups. The result of the Acute toxicity study as per OECD guideline- 423 indicates that the LD50 of CN is more than 2000mg/ kg and the test drug CN comes under the category- 5 according to the Globally harmonized system of classification and labelling of chemicals.

Acute Toxicity Study	
Analysis	**Group I**
Consistency	Soft- Fatty
Shape	Oblong
Colour	Dark Green
Mucous Shedding	Absence
Blood Cells	Absent
Signs of Infection	None Observed

Sub acute toxicity study

There were no treatment related toxic signs and mortality observed in both male and female rats treated at mid (200 mg/kg) and high dose (400mg/kg) levels. There were no significant changes in clinical sign, body weight, food and water intake (Table 1,2,3) of both control and drug treated groups. The haematological (Table 4) and biochemical parameters (Table 5) are within the normal range of rats in experimental groups.[8] There were no abnormalities in the gross and histo-pathological studies. It revealed that the NOAEL (No-Observed Adverse Effect Level) of CN is greater than the 400 mg/ kg/ day in rats, hence, it can be concluded that the oral administration of CN is safe.

Fecal Pellet Analysis

Methodology

Rats of control and treatment group were allowed to explore to open field on clean and sterile Stainless steel tray. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc.

Sub-Acute Toxicity Study			
Analysis	**Group I**	**Group II**	**Group III**
Consistency	Soft	Soft	Soft – steatorrhea
Shape	Oblong	Round ended	Round ended
Colour	Greenish	Brown	Brown
Mucous Shedding	Absence	Absence	Absence
Blood Cells	Absent	Absent	Absent
Signs of Infection	None Observed	None Observed	None Observed

Muscle Grip Strength Analysis

The grip strength test is a simple non-invasive method designed to evaluate rat muscle force in vivo. Rats of control and drug treated group was allowed to hold the pull bar with both the hind limbs firmly then the animal was gently pulled back with the tail until the animal lost the grip toward the bar. The procedure was repeated to get the average value. Muscle gripness of the drug treated group was compared to that of the control rat to ensure the change in coordination.

Metabolic Cage for Urine Collection

Rat of control and treatment group was placed individually in metabolic cage with free access to feed and water. Urine dropping from the animal was collected using specialized wire mesh system fixed at the base of the cage having provision to trap the fecal pellet mixed with urine sample. The collected urine sample was subjected to analysis with respect to colour, pH, glucose, ketone bodies, pus and blood cells.

Assessment of clinical signs in rats treated with *Chitramutti Nei* on Acute toxicity study

Acute	
Parameter	**Group I**
Clinical Signs Parameters for the duration of 14 days	Test Drug 2000mg/kg
Number of animals observed	6 Female
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Mild
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Color	Normal
Stereotype behavior	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behavior	Normal
Giat Balancing	Normal
Freezing Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	6
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

Quantitative data on the body weight of rats treated with *Chitramutti Nei* in Acute toxicity study

Group I	Before Treatment Weight in Gms	After Treatment Weight in Gms
Mean	177.7	186.2
Std. Deviation	6.89	9.847
Std. Error	2.813	4.02

Values are mean ± S.D (n = 6 per group). Statistical significance carried out using one way ANOVA followed by Dunnett's test.

Table 1 Assessment of clinical signs in rats treated with *Chitramutti Nei* on Sub-Acute toxicity study

Sub Acute			
Parameter	**Group I**	**Group II**	**Group III**
Clinical Signs Parameters for the duration of 28 days	Control	Test Drug 200mg/kg	Test Drug 400mg/kg
Number of animals observed	3 Males and 3Females	3 Males and 3Females	3 Males and 3Females
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Mild	Moderate
Touch Response	Normal	Normal	Normal
Response to Sound	Normal Response	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response	Normal Response
Mobility	Normal Response	Normal Response	Normal Response
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Giat Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	6	7	7
Urine -Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

Table 2 Effect of *Chitramutti Nei* on Body weight of Rats in Sub-acute toxicity study

Group I	**Before Treatment Weight in Gms**	**After Treatment Weight in Gms**
Mean	184	195.5
Std. Deviation	5.177	5.958
Std. Error	2.113	2.432
Group II	**Before Treatment Weight in Gms**	**After Treatment Weight in Gms**
Mean	180.5	191.2
Std. Deviation	7.893	10.44
Std. Error	3.222	4.262
Group III	**Before Treatment**	**After Treatment Weight in Gms**
Mean	184.3	194.2
Std. Deviation	6.563	6.735
Std. Error	2.679	2.75

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 3 Quantitative data on the food and water intake of rats treated with *Chitramutti Nei* for 28 days in Sub-acute toxicity study

GROUP I	**Food intake**	**Water intake**
Mean	17.42	22.25
Std. Deviation	3.573	6.437
Std. Error	1.787	3.219
GROUP II	**Food intake**	**Water intake**
Mean	19.25	32.83
Std. Deviation	1.032	2.253
Std. Error	0.5159	1.126
GROUP III	**Food intake**	**Water intake**
Mean	16.75	31
Std. Deviation	1.813	2.126
Std. Error	0.9065	1.063

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 4 Effect of *Chitramutti Nei* on Haematology profile of rats in sub-acute toxicity study.

GROUP I	WBC count (×103 µl)	RBC (×10 ^{6} µl)	PLT (×10 ^{3} µl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HGB (g/dl)
Mean	9.933	6.6	679.8	58.83	19.22	32.75	13.27
Std. Deviation	1.483	1.103	174.3	6.218	2.232	1.299	1.089
Std. Error	0.6053	0.4502	71.15	2.538	0.9112	0.5303	0.4447
GROUP II	**WBC count (×103 µl)**	**RBC (×10 ^{6} µl)**	**PLT (×10 ^{3} µl)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HGB (g/dl)**
Mean	8.85	6.517	1008	62.48	19.57	31.57	13.05
Std. Deviation	0.8939	0.9196	244.9	5.319	1.99	1.372	1.495
Std. Error	0.3649	0.3754	100	2.172	0.8123	0.5602	0.6103
GROUP III	**WBC count (×103 µl)**	**RBC (×10 ^{6} µl)**	**PLT (×10 ^{3} µl)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HGB (g/dl)**
Mean	9.35	6.183	1015	62.25	18.07	32.77	13.90
Std. Deviation	2.651	1.026	224.8	4.567	1.802	1.581	1.556
Std. Error	1.082	0.4191	91.76	1.864	0.7356	0.6453	0.6351

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Chitramutti Nei* on Haematology profile of rats in sub-acute toxicity study.

GROUP I	Lymph (%)	Mon (%)	Neutrophils 10^{3}/mm3	Eosinophils (%)	Basophils (%)	MPV (fl)
Mean	80.92	3.6	2.5	1.667	0.1667	5.833
Std. Deviation	6.151	0.9033	0.6033	0.2251	0.4082	1.14
Std. Error	2.511	0.3688	0.2463	0.09189	0.1667	0.4652
GROUP II	**Lymph (%)**	**Mon (%)**	**Neutrophils 10^{3}/mm3**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Mean	76.93	3.45	2.15	1.55	0.1667	6.167
Std. Deviation	5.562	1.097	0.5891	0.3271	0.4082	1.5
Std. Error	2.271	0.4478	0.2405	0.1335	0.1667	0.6125
GROUP III	**Lymph (%)**	**Mon (%)**	**Neutrophils 10^{3}/mm3**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Mean	74.67	3.65	2.617	1.383	0.3333	5.967
Std. Deviation	5.923	1.071	0.736	0.2994	0.5164	1.289
Std. Error	2.418	0.4372	0.3005	0.1222	0.2108	0.5264

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 5 Effect of *Chitramutti Nei* on Serum Bio-chemistry profile of rats in sub-acute toxicity study

GROUP I	Blood sugar (mg/dl)	BUN (mg/dl)	Serum creatinine (mg/dl)	Serum total cholesterol (mg/dl)	Serum triglycerides level (mg/dl)	Serum HDL cholesterol (mg/dl)	Serum LDL cholesterol (mg/dl)	Serum VLDL cholesterol (mg/dl)
Mean	77	18.83	0.6833	117	74.33	69.17	31.33	16.45
Std. Deviation	6.066	2.317	0.1722	4.942	5.888	3.545	5.007	2.971
Std. Error	2.477	0.9458	0.07032	2.018	2.404	1.447	2.044	1.213
GROUP II	**Blood sugar (mg/dl)**	**BUN (mg/dl)**	**Serum creatinine (mg/dl)**	**Serum total cholesterol (mg/dl)**	**Serum triglycerides level (mg/dl)**	**Serum HDL cholesterol (mg/dl)**	**Serum LDL cholesterol (mg/dl)**	**Serum VLDL cholesterol (mg/dl)**
Mean	78.67	17.33	0.75	108.7	77.67	60.67	32.17	15.88
Std. Deviation	7.528	4.367	0.1871	7.913	7.339	5.502	9.786	2.003
Std. Error	3.073	1.783	0.07638	3.23	2.996	2.246	3.995	0.8179
GROUP III	**Blood sugar (mg/dl)**	**BUN (mg/dl)**	**Serum creatinine (mg/dl)**	**Serum total cholesterol (mg/dl)**	**Serum triglycerides level (mg/dl)**	**Serum HDL cholesterol (mg/dl)**	**Serum LDL cholesterol (mg/dl)**	**Serum VLDL cholesterol (mg/dl)**
Mean	86.83	16.17	0.8333	115.1	74.83	64.67	32.83	17.55
Std. Deviation	13.92	3.869	0.216	7.234	7.36	7.367	10.15	3.257
Std. Error	5.683	1.579	0.08819	2.953	3.005	3.007	4.143	1.33

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table- 6 Effect of *Chitramutti Nei* on Serum Bio-chemistry profile of rats in sub-acute toxicity study

GROUP I	Serum total protein (g/dl)	Serum albumin (g/dl)	(AST) (IU/ml)	(ALT) (IU/L)	(ALP) (IU/L)
Mean	6.35	3.6	102.3	36.5	112
Std. Deviation	0.9995	0.08944	13.87	7.714	15.47
Std. Error	0.408	0.03651	5.661	3.149	6.314
GROUP II	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**(AST) (IU/ml)**	**(ALT) (IU/L)**	**(ALP) (IU/L)**
Mean	6.467	3.667	100.2	28	137.7
Std. Deviation	0.7062	0.4412	16.24	8.149	18.52
Std. Error	0.2883	0.1801	6.63	3.327	7.562
GROUP III	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**(AST) (IU/ml)**	**(ALT) (IU/L)**	**(ALP) (IU/L)**
Mean	6.7	3.883	96.17	27.17	117.5
Std. Deviation	0.743	0.4708	13	8.704	23.56
Std. Error	0.3033	0.1922	5.307	3.554	9.619

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 7: Quantitative data on absolute organ weight of rats treated with *Chitramutti Nei* for 28 days in Sub-acute toxicity study.

GROUP I	HEART (gms)	LIVER (gms)	KIDNEY (gms)	SPLEEN (gms)	BRAIN (gms)	LUNG (gms)	STOMACH (gms)	TESTES (gms)	UTERUS & OVARY (gms)
Mean	0.7683	5.948	1.617	0.5667	1.483	1.6	1.15	1.833	0.8667
Std. Deviation	0.05636	0.9834	0.2137	0.1633	0.1472	0.3162	0.501	0.4933	0.4041
Std. Error	0.02301	0.4015	0.08724	0.06667	0.06009	0.1291	0.2045	0.2848	0.2333
GROUP II	**HEART (gms)**	**LIVER (gms)**	**KIDNEY (gms)**	**SPLEEN (gms)**	**BRAIN (gms)**	**LUNG (gms)**	**STOMACH (gms)**	**TESTES (gms)**	**UTERUS & OVARY (gms)**
Mean	0.5767	5.183	1.6	0.6167	1.45	1.467	0.7667	2.033	0.7667
Std. Deviation	0.155	0.9404	0.1414	0.1472	0.1871	0.3077	0.1211	0.3215	0.3786
Std. Error	0.06328	0.3839	0.05774	0.06009	0.07638	0.1256	0.04944	0.1856	0.2186
GROUP III	**HEART (gms)**	**LIVER (gms)**	**KIDNEY (gms)**	**SPLEEN (gms)**	**BRAIN (gms)**	**LUNG (gms)**	**STOMACH (gms)**	**TESTES (gms)**	**UTERUS & OVARY (gms)**
Mean	0.6583	5.718	1.567	0.6333	1.55	1.483	1	3.167	0.9333
Std. Deviation	0.2088	1.055	0.2338	0.216	0.1643	0.2401	0.3521	0.3215	0.4933
Std. Error	0.08526	0.4309	0.09545	0.08819	0.06708	0.09804	0.1438	0.1856	0.2848

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females) for Heart, Liver, Kidney, Brain, Spleen, Lung, Stomach. Values are mean ± S.D (n = 3 per group per sex) for testes , ovary and uterus for Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Discussion

Herbal medicines have attained greater importance as an alternative to clinical therapy and the demand for these remedies has currently increased.[9] Many screening methods are employed to determine the safety and efficacy of these herbal medicines and also to establish the active component of the herbal products.[10]

In acute toxicity study of CN were administered in single oral doses administration as per OECD 423 guidelines. There was no mortality and signs of toxicity were observed in CN treated animals throughout the study period, the LD50 of CN is more than 2000mg/ kg and it comes under the category- 5 according to the globally harmonized system of drugs. Therefore it can be concluded that the single administration of CN is non-toxic and safe for oral administration.

Examination of clinical signs plays a important role in toxicological studies.[11] mortality and morbidity were recorded throughout the study period. In repeated dose 28 days study as per OECD guidelines 407 revealed there were no mortality and morbidity in two different dose levels (mid dose (200 mg/kg/day) & high dose (400 mg/kg/day)) of CN.

There was a normal water intake and food consumption (Table 2) and there was an obvious weight gain (Table 1) in the CN treated groups when compared with control, it revealed that the test drug did not adversely affect the basic metabolic functions of the experimental animal.

Clinical biochemistry and haematological parameters plays an important role to identify the toxicity induced by drugs.[12] Analysis of haematological parameters (Table 4) showed no significant changes in CN treated groups compared with control, it indicates it is the safe formulation and no pathological changes in haemopoietic system. With the exception of a transient increase in hemoglobin and WBC count there were no significant alterations in the hematological parameters. This increase in the haemoglobin level might be due to the increased absorption of iron by vitamin C in CN and the increase in the WBC level may indicate the impact of CN in boosting the immune system of treated groups.

Lipid profiles such as HDL, LDL, VLDL, TGL, Total Cholesterol did not show any significant changes. The main product of protein metabolism is urea and an increased level of urea in the blood is an indicator of renal impairment. The present study showed no significant changes pertaining to renal parameters. Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) which is the indicators of hepatocellular injury also did not show any significant alterations in the *Chitramutti Nei* treated groups and control groups (Table-5& 6).

The histopathological studies revealed no significant weight changes and normal architectural changes in the vital organs such as heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary suggesting that the preparation is devoid of serious organ degenerative potential both dose levels.

From the above results, it can be indicated that the No Observed Adverse Effect Level of *Chitramutti Nei* was greater than 400mg/kg/day.

Conclusion

Through this acute and sub acute toxicity study, the drug *Chitramutti Nei* (CN) a traditional Siddha herbal formulation was found to be safe on both doses of 200 & 400 mg/kg and the LD50 was found to be higher than 2000 mg/kg. Since there are no toxic effects produced by the drug, further clinical studies would be conducted to prove the efficacy of *Chitramutti Nei* (CN) in the treatment of anaemia.

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