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RESEARCH ARTICLE
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SUB CHRONIC TOXICITY OF UPPU CHENDURAM -II -A SIDDHA HERBOMINERAL FORMULATION IN WISTAR RAT MODELS

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ABSTRACT

Among the various toxicity tests conducted, Sub-chronic toxicity studies play a crucial role in assessing the long-term health effects of these substances. Sub-chronic testing provides valuable insights into the immediate or short-term effects of a substance, and the consequences of exposure. While Uppu Chenduram II has been prescribed in classical Siddha text since time immemorial for acid peptic disease it lacks scientifc data on its safety concerns. Since this medicine requires to be taken atleast for 30-90 days, the testing for subchronic toxicity becomes crucial to evidence its safety Therefore the present study aimed to detect the non-observed-adverse-effect level (NOAEL) in sub chronic oral toxicity study of Siddha preparation *Uppu chenduram*-II (UC-II) in Wistar rats. Experimental animals were divided into four groups of 5 rats were placed in cages. Groupt-1 served as control (i.e. the rats were fed without UC-II), then the groups II to IV were daily fed by oral administration of Siddha preparation Uppu chenduram-II at different doses (200, 400 & 600 mg/kg) for 90 days after which the subchronic toxicity profiling was determined. The study results revealed that *Uppu chenduram*-II caused no adverse effect in any of the tested parameters and the histopathological investigations of the vital organs also produce any toxic changes confirming the safety of the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) in the vital organs.

Keywords: Uppu Chenduram, Siddha, Subchronic toxicity, Safety, Herbal medicine

INTRODUCTION.

Most of today's commonly used medicnes are from Herbal sources. About 80% of the world's population rely on herbal /traditional medicines for as the primary form of medicine [1-2]. Usually, a specific part of the plant (root, leaves, fruit, flowers, and seeds) is used in traditional preparations or

as pure active principles formulated into a suitable preparation. Plant derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide[3-5]. The Siddha system of medicine is the oldest traditional treatment system generated from Dravidian culture that flourished during the Indus Valley civilization[6]. The basis of Siddha science is understanding that earth, air, water, fire and ether correspond to the five senses of the human body are fundamental to all living things[7] Siddha herbomineral drug *Uppuchenduram* provides insights to the fact of the possible target action in claiming the traditional indications of this formulation against all kinds of ulcer(*Kunmam*)[8].

Uppu Chendhuram, a herbo-mineral formulation used for treating indigestion and all types of ulcers as indicated in the Classical text "*Siddha Vaidhiya Thirattu".* The ingredients of *Uppu Chendhuram* include *sotruppu* (Sodium Chloride) and *Agayathaamarai* extract (Pistia stratiotes) [9]

Toxicity testing employs a wide range of tests in different species of animals with long-term administration of the drug, regular monitoring of physiological, biochemical abnormalities and detailed post-mortem examination at the end of the trial to detect gross or histological abnormalities[10]. All toxicity study is supported by clinical analysis, autophic analysis, haematological and haematochemical analysis, histopathological analysis and statistical presentation and data interpretation. Toxicity testing is of the following types: acute toxicity studies, sub-acute toxicity studies and chronic toxicity studies.[11]. To ensure the efficacy of *Uppu Chendhuram*, a comprehensive subchronic toxicity study was performed to reveal the drug's long-term effect on the body organs of experimental rats, which allows the drug to provide crucial safety data that supports the rationale for the proposed dosing regimens and duration of use in future human clinical trials.

Materials and methods Preparation of Uppuchenduram

5 Palam of Sotruppu (Sodium chloride) is to be taken and grounded with ¼ padi of Agayathamarai extract (Pistiastratoides) .The mixture is made into pellets (villai) .and are tightly packed in the mudpots sealed with clay cloth layers and undergo heating process in well designed pits made out of special fire bricks. and burnt using 10 cow dung cakes. A similar manner of incineration to be carried out by increasing the cow dung cakes at each time up to 20 cakes.

Indication:1-2 rice pills for all kinds of acidity, Acid peptic disease

Experimental animals

This investigation was completed in 90 days according to the OECD guidelines 407 (OECD 407, 2008).Experimental animals Rats used in this experiment were obtained from the Department of pharmacology, K.M.College of Pharmacy, Madurai. They were caged in a hygienic, conducive habitat with proper lighting. The rats weighed between 180 and 200 g. The rats were fed orally with rat pelleted feed (Hindustan agro feeds, Bangalore), they had access to dirt-free drinking water and they were housed in steel cages, in compliance with the National Research Council guidelines for the care and use of laboratory animals (National Research Council, 2011).

Grouping of animals

Experimental animals were divided into four groups of 5 rats were placed in cages. Set 1 served as control (i.e. the rats were fed without siddha preparation), then the groups 2to 4 were daily fed by oral administration of Siddha preparation Uppu chenduram-II at different doses (200, 400 & 600 mg/kg) for 90 days. On day 90, the rats were anaesthetized using ether after fasting for the night while blood samples were taken for biochemical and haematological analyses using both EDTA and plain vials while the brain,heart,kidney and liver were harvested for histological assessment.

Ethical issues

The research procedures for animal handling were approved IAEC/BHUVANESWARI T/TNMGRMU/PH.D/M.D(S)/KMCP/138/2021- 22 by the Institutional Animal ethical committee of the K.M.College of Pharmacy, Madurai.

Relative organ and body weights study

The changes in body weights were recorded on a weekly basis, while the organs (the liver, spleen, kidneys, brain and heart) were weighed using standard weighing balance to calculate relative organ weight for the different sets on the sacrifice day.

Relative organ weight (%) = [Absolute weight of organ (g)/weight of rat on sacrifice day (g)] x100

Hematological analysis

The indices analysed in the blood samples included haematocrit (HCT), corpuscular volume (CV), erythrocyte count, lymphocytes (LYM), neutrophils (NEU), monocytes (MONO), thrombocyte count, basophils (BASO), leucocyte count (WBC) were performed by means of an automated analyzer.

Assessment of serum biochemical parameters

Blood for biochemical analysis were gently placed in plain bottles to avoid haemolysis of the blood cells. Blood serum was obtained by centrifugation of the blood sample. Biochemical studies were carried out using standard methods for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and conjugated bilirubin, total protein, albumin, urea and creatinine. The serum electrolytes (Sodium, potassium, chloride and bicarbonate ions) were estimated using an automated ion selective electrode machine (Audicom electrolyte analyser).

Lipid profile

Statistical analysis

The results of the study are expressed as the mean ±S. E. M. The results were analysed using Graph Pad Prism version 3 software. Comparison in all the groups was made using one-way analysis of variance (ANOVA) followed by Newmann keuls multiple range tests. Differences were considered significant at $P<0.05$.

RESULTS Subchronic toxicity study

The sub-chronic toxicity study of the tested Siddha preparation Uppu chenduram-II was determined as per OECD guideline 407. All study animals given Siddha preparation Uppu chenduram-II daily at all study doses (200,400&600 mg/kg) survived the entire 90-day period. No signs of toxicity were observed in the Siddha preparation Uppu chenduram-II treated group compared to the control group.The following results of the study are tabulated from Table-1 to Table-6.

Effects of the Siddha preparation Uppu chenduram-II on food and water intakes

Table1-Effects of the Siddha preparation Uppu chenduram-II on food and water intakes in subchronic toxicity study.

Treatment	Average food intake (g/d)	Average water intake(ml/d)
Normal control Normal saline (10ml/kg)	14.50± 1.45	18.30 ±2.50

Uppu chenduram-II (200mg/kg)	18.10± 1.75	24.35 ±2.80
Uppuchenduram-II (400mg/kg)	17.85± 1.60	19.85 ±2.75
Uppuchenduram- II (600mg/kg)	17.60± 1.50	22.45 ±2.95

Values are expressed as mean ± standard deviation; n = 5. No significant difference between Siddha preparation Uppu chenduram-II treatment groups and control group ($P > 0.05$)

Table 1 depicts the effects of the Siddha preparation Uppu chenduram-II on the food and water intakes in sub chronic treatment. The single daily administration of the Siddha preparation Uppu chenduramII at study doses (200,400 and 600 mg/kg) for 90 d caused no significant changes ($P > 0.05$) in food and water intakes when compared with the control group.

Table-2-Effects of the Siddha preparation Uppu chenduram-II on the relative weight of organs in rats

Treatment	Relative organ weight (%)					
	Heart	Liver	Lungs	Kidney	Spleen	Brain
Normal control Normal saline (10ml/kg)	0.88±0.06	8.22±0.85	1.20±0.40	2.32±0.85	0.68±0.27	1.24±0.09
Uppu chenduram II (200mg/kg)	0.91± 0.11	9.10±1.08	1.14±0.34	2.15±0.68	0.61±0.24	1.40±0.15
Uppu chenduram-II (400mg/kg)	0.93± 0.15	9.02±1.00	1.28±0.45	2.20±0.77	0.65 ±0.27	1.33±0.13
Uppuchenduram-II (600mg/kg)	0.90± 0.10	9.24±1.24	1.24±0.42	2.24±0.74	0.72 ±0.28	1.28 ±0.10

Values are expressed as mean ± standard deviation; n = 5. No significant difference between Siddha preparation Uppu chenduram-II treatment groups and control group ($P > 0.05$)

Daily administration of the Siddha preparation *Uppu chenduram-II* for 90 days did not cause any significant alteration ($P > 0.05$) in organ weights in the experimental groups relative to control (Table 2). The results revealed that the vital organs such as liver, kidneys, heart, lungs, brain and spleen we not adversely affected throughout the treatment period (90d).

Table- 3 Effects of the Siddha preparation Uppu chenduram-II on the bodyweights.

Treatment	**Body weight (Gm)**				
	0 Days	**15 Days**	**30 Days**	**60 days**	**90 Days**
Normal control Normal saline (10ml/kg)	184.10±4.15	188.75 ±4.70	193.40± 4.90	210.55± 4.90	225.15 ±5.20

Uppu chenduram-II (200mg/kg)	195.85±4.50	199.35± 4.80	207.40±5.10	222.50 ±5.45	240.50 ±5.75
Uppuchenduram-II (400mg/kg)	188.95±4.30	193.40± 4.60	199.15±4.85	230.45 ±5.40	255.30 ±5.55
Uppuchenduram-II (600mg/kg)	190.45±4.35	195.75± 4.75	201.45±4.85	225.80 ±5.35	245.45 ±5.75

Values are expressed as mean ± standard deviation; $n = 5$. No significant difference between Siddha preparation Uppu chenduram-II treatment groups and control group ($P > 0.05$)

Table-4-Effects of the Siddha preparation Uppu chenduram-II (UC) on haematological parameters of treated rats

Treatment	**Control**	**UC II (200mg/kg)**	**UC-II (400 mg/kg)**	**UC-II (600mg/kg)**
WBC($\times 10^9$/L)	9.15 ± 1.05	9.28 ± 1.14	9.45 ± 1.22	9.58 ± 1.34
RBC ($\times 10^{12}$/L)	7.75 ± 0.38	7.92 ± 0.44	8.08 ± 0.51	8.7 ± 0.56
Hb (g/dL)	15.35 ± 1.35	15.48 ±1.42	14.75± 1.20	15.65± 1.45
MCV (fL)	68.65 ± 2.85	66.70 ± 2.60	65.10 ± 2.35	65.75 ± 2.50
MCH (pg)	18.75 ± 1.40	19.20 ±1.65	19.45 ± 1.80	19.15 ± 1.55
PCV ($\times 10^9$/L)	755.20±20.25	768.35 ± 22.30	772.40 ± 22.65	760.30 ± 22.35
Neutrophils ($\times 10^9$/L)	0.42 ± 0.10	0.58 ± 0.14	0.62 ± 0.20	0.68 ± 0.24
Lymphocytes (10^9/L)	6.34 ±0.65	6.48 ± 0.95	6.42 ± 0.85	6.53 ± 0.90
Haematocrit (L/L)	0.60 ± 0.10	0.54 ± 0.08	0.56 ± 0.09	0.61 ± 0.13

Values are expressed as mean ± standard deviation; $n = 5$. No significant difference between Siddha preparation Uppu chenduram-II treatment groups and control group ($P > 0.05$)

Table-5 Effects of the Siddha preparation Uppu chenduram-II on some serum biochemical parameters in sub-chronic toxicity study of treated rats

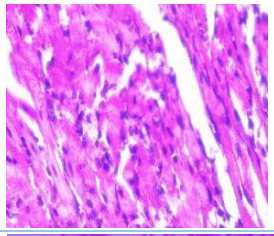
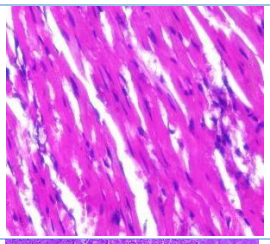
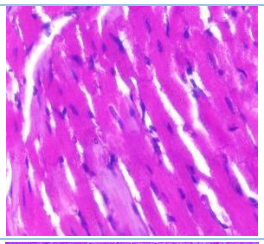
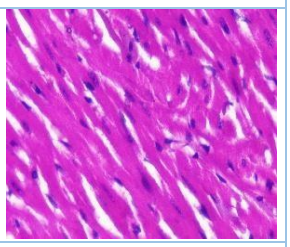
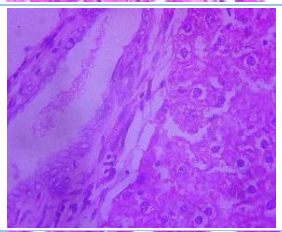
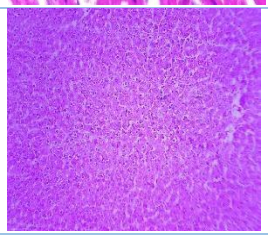
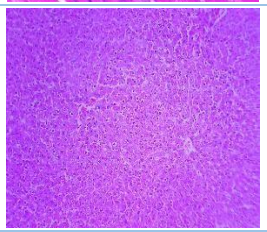
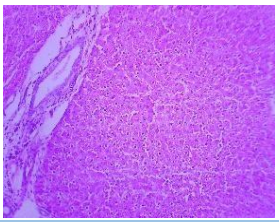
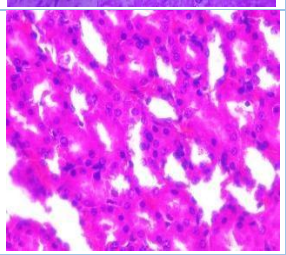
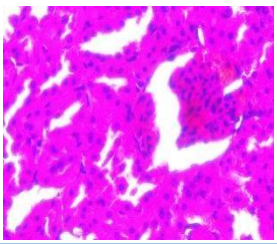
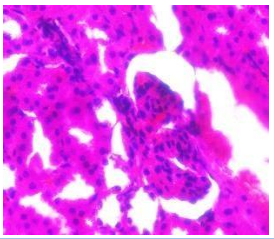
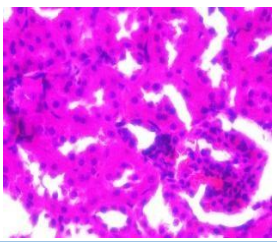
Treatment	**Control**	**UC-II 200mg/kg**	**UC-II 400 mg/kg**	**UC -II 600mg/kg**
Sodium (mmol/L)	134.50 ± 4.45	142.65 ± 5.10	140.20 ± 4.85	145.80 ± 5.50
Chloride (mmol/L)	107.40 ± 3.75	104.20 ± 3.45	105.75 ± 3.60	108.80 ± 3.90
Urea (mmol/L)	4.40 ± 0.35	4.75 ± 0.50	4.60 ± 0.40	4.85 ± 0.65
Creatinine (µmol/L)	32.32 ± 1.75	31.80 ± 1.50	30.80 ± 1.35	33.20 ± 1.90
Glucose (mmol/L)	5.15 ± 0.60	4.95 ± 0.45	5.20 ± 0.70	5.35 ± 0.75
Calcium (mmol/L)	2.58 ± 0.18	2.60 ± 0.24	2.65 ± 0.28	2.50 ± 0.15
Magnesium(mmol/L)	0.98 ± 0.08	1.08 ± 0.18	1.22 ± 0.23	1.18 ± 0.20
Uric acid (mmol/L)	0.18 ± 0.05	0.24 ± 0.10	0.20 ± 0.08	0.26 ± 0.12

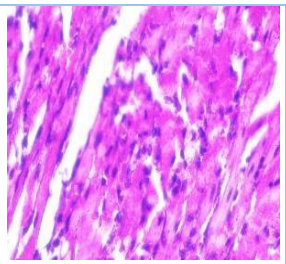
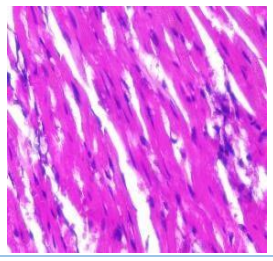
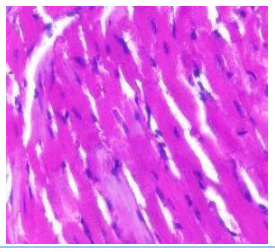
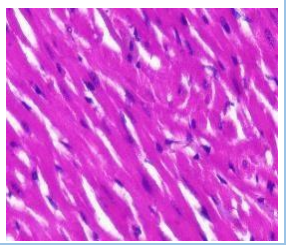
Total protein (g/L)	52.45 ± 2.85	53.45 ± 2.90	55.20 ± 3.15	54.20 ± 3.22
Albumin (g/L)	19.85± 0.45	19.40 ± 0.35	19.60 ± 0.40	19.55 ± 0.50
Total bil (µmol/L)	16.75 ± 3.65	16.30 ± 3.55	15.90 ± 3.40	16.15 ± 3.05
ALT (U/L)	60.75 ±3.20	61.60 ± 3.35	62.80 ± 3.50	63.05 ± 3.70
AST (U/L)	162.30 ±4.30	158.50 ±4.15	160.45 ±4.20	157.20 ±4.10
ALP (U/L)	252.40 ±5.50	258.20 ±5.75	256.50 ±5.65	265.35 ±5.80
Total cholesterol(mmol/L)	1.08 ± 0.08	1.15 ± 0.18	1.18 ± 0.20	1.22 ± 0.24
TAG (mmol/L)	2.30 ± 0.45	1.85 ± 0.30	1.95 ± 0.40	1.90 ± 0.36
HDL cholesterol(mmol/L)	0.74 ± 0.08	0.79 ± 0.11	0.85± 0.14	0.88 ± 0.18

Values are expressed as mean ± standard deviation; $n = 5$. No significant difference between Siddha preparation Uppu chenduram-II treatment groups and control group ($P > 0.05$)

Histopathology

Histopathological examinations were performed on the liver, kidney,brain,pancreas and heart, to assess whether or not organs or tissues had been damaged. We found that none of the organs of the rats given daily Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) showed any morphological alterations or abnormalities under the light microscope.

Organs	**Normal control**	Uppu Chenduram-II **(200mg/kg)**	Uppu Chenduram-II **(400mg/kg)**	Uppu Chenduram-II **(600mg/kg)**
Pancreas				
Liver				
Kidney				

Heart				

DISCUSSION

The principal aim of evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effect and to establish the exposure level at which this effect is observed [6]. The results of the acute toxicity study indicate that the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) administered through oral route to rats, using the up and down method of acute toxicity testing did not produce any sign of toxicity and death in the animals. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD50 > 2000 to 5000 mg/kg are categorised as unclassified or category 5 (Organization for Economic Development, 2001)[12].

Acute toxicity data are usually of limited clinical application. Therefore, sub-chronic toxicity study was carried out. Substances administered in chronic disease conditions may need repeated dosing toxicological evaluation (Sub-chronic toxicity study) since daily use may result in accumulation in the body with gradual effects on tissues and organs[13][14]

The food and water intake of experimental animals was determined periodically throughout the study. From these determinations, the mean individual daily food and water consumption and its efficiency were calculated. Body weights of all animals were observed on 90-day basis individually, and the percentage weight gain of the treated animals was compared with that of the control group to observe clinical signs of toxicity, just before exposure, throughout the study [12].Table 1 provides that *Uppu Chenduram-II* may have an effect on food and water intake, with varying doses showing different effects[13]. The findings include that the control group has the least amount of food and water intake, while the *Uppu Chendhuram* at different doses (200mg/kg, 400mg/kg, 600mg/kg) showed increased food intake when compared to the control group. However, the largest dose (600mg/kg) has a slightly lesser food intake (17.60±1.50) when compared to the lower dosages. But the changes in food and water intake did not alter the relative organ weight of vital organs.

The wellness status of animals is hinged on changes in body weight [15]. After 90 days of treatment, all the animals exhibited a steady increase in body weight. It indicates that the daily intake of the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) did not alter food intake. Furthermore, it possibly shows that weight gain and appetite stability were not impeded by the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) during the exposure period. In the subchronic toxicity study, no deaths were recorded after oral administration of 200, 400 and 600 mg/kg for 90 d. The animals did not present any behavioral changes when subjected to Hippocratic screening for this experimental period (90 d). There were no significant changes in food or water consumption among treated rats throughout the 90-day study, which is an indicator that the diet and water were well tolerated by the animals. Again, in toxicity studies, organ weight changes are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances and target organ injury[15]. An increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ[16]. While organ weights provide useful signals indicating test article-related effects, organ weight data must be interpreted in an integrated fashion with gross pathology, clinical pathology, and histopathology findings[17].According to Raina et al. and Cajuday and Pocsidio[18][19],the weights of the organs are markers of pathological and physiological wellness

status of animals. Changes in organ weights are hallmarks of toxicity in experimental animals, which are determined by toxicity tests. [19].

On completion of the 90th day, both the control and drug-treated animals were deprived of food overnight and sacrificed by cervical decapitation. Vital organs like the liver, kidney, lung, spleen, and heart of each animal were separated after thorough perfusion of the organs with neutral saline and pressed with the help of tissue paper to remove any moisture. The isolated organs were observed for morphological changes, such as the presence of any kind of lesions, etc., and the individual organs from each animal were weighed.

The toxic effect of ingested herbal remedies in the body is most likely to be felt by important organs such as the spleen, heart, liver and kidneys because of the vital roles that they play in the body[20]. The liver and kidneys are major targets of xenobiotic action, with the liver being the main organ for xenobiotic biotransformation, while the kidney serves as excretory organ of xenobiotics[21]

The results were compared to those of the control animals.[22] Daily administration of the Siddha preparation Uppu chenduram-II for 90 days did not cause any significant alteration ($P > 0.05$) in organ weights in the experimental groups relative to the control (Table 2). The results revealed that the relative weight of vital organs such as liver, kidneys, heart,lungs, brain and spleen were not adversely affected throughout the treatment period (90d). With the p-value> 0.05, the Siddha formulation *Uppu Chendhuram* doesn't indicate toxicity on organ weight indicating no catabolic activity.

Body weight changes are considered as the perceptive and analytical marker for the first sign in the toxicity studies when exposed to toxic substances [23]. In this study animals were found to show an increase in body weight in chronic toxicity study. Hence the interpretation of results support normal food consumption throughout the toxicity study period. Moreover the effects of the Siddha preparation Uppu Chenduram II on the body weights shows a gradual increase in body Weight with increase in dosage this shows a positive sign as the herbomineral drug does not affect the body weight of study animal.

The wellness status of animals is hinged on changes in body weight[15] After 90 d of treatment, all the animals exhibited a steady increase in body weight. It indicates that the daily intake of the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) did not alter food intake.

Furthermore, it possibly shows that weight gain and appetite stability were not impeded by the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) during the exposure period. Sub-chronic toxicity testing is useful in assessing target organ and haematological or biochemical effects of Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) since these effects are usually not observable in acute toxicity testing. It is also essential in establishing human safety especially in the development of pharmaceuticals.

Analysis of haematological parameters are used to study the extent of toxicity of drug substances including plant extracts. Haematopoiesis is the process of blood cell formation. Changes in the haematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies[24]. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet)[25].

In this study, administration of at a dose of (200,400 and 600 mg/kg) in rats for a period of 90 days produced no significant change in all blood parameters except an increase in lymphocytes and mean

platelets volume. Lymphocytes are dynamic cells and mediate immune response to foreign substances[26]. They also produce antibodies enabling the destruction of intracellular microbes and cancer cells[27]

An important index of physiological and pathological status in any toxicity study is the Hematopoietic system. No abnormality was observed in hematopoietic function for the study groups compared with control groups indicating the drug is safe[15].Siddha preparation Uppu chenduram-II did not induce significant haematological toxicity in rats at doses of 200mg/kg, 400mg/kg, and 600mg/kg. Lack of significant differences in haematological parameters between treatment groups and the control group indicates that Uppu chenduram-II may not have a toxic effect on the haematological system.The stability of parameters like WBC, RBC, Hb, and haematocrit suggests that Uppu chenduram-II may not cause bone or haematological disorders.

The role of liver and kidney functions are important for survival of animals. Their functionality can be measured by serum biochemical analysis, which are crucial in the toxicological evaluation of xenobiotics[28]. Serum liver function tests provide information about the status of the liver. The liver enzymes (aminotransferases; ALT and AST) describe its cellular integrity, while albumin and total protein levels describe its functionality[29] AST and ALT are principally produced by the liver cells and any assault to the liver may lead to an increase in the serum level of these enzymes. High levels of liver enzymes are signs of hepatocellular toxicity[30], whereas a decrease may indicate enzyme inhibition[31]

However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles [32]. In this study, there was no significant changes in the ALT of treated rats.The functionality of the liver was assessed by the serum total protein, bilirubin and albumin. A reduction in serum levels of total proteins, bilirubin and albumin depicts reduced synthetic function, which is evident in liver damage or diseases. An increase in these parameters is usually seen in cancerous conditions, or following high protein diet[33]. Our study showed a non-significant change in total protein and albumin serum levels from the control group. Urea, uric acid and creatinine are the basic end products of protein metabolism and must be removed usually to ensure protein metabolism in the body[34]. A high serum level of urea indicates that the kidneys may not be working properly, or that the animal is dehydrated whereas, low urea levels are seen in acute liver failure or overhydration clearance, an indicator of glomerular filtration rate is used for assessing kidney function[35]. Our Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) did not cause any significant change in the creatinine levels when compared with the control suggesting that the at a dose of (200,400 and 600 mg/kg) may not be toxic to the kidney. [22]

Conclusively, Sodium, chloride, calcium, and magnesium levels remained within normal ranges**. .** In the renal function Urea, creatinine, and uric acid levels did not show significant changes, indicating no adverse effects on kidney function. Metabolic Parameters like Glucose levels remained stable, indicating no impact on glucose metabolism. Liver Function markers that is total protein, albumin, and total bilirubin levels did not show significant alterations, indicating no adverse effects on liver function**.**

Histology of the liver, kidney,brain,pancreas and heart in the rats did not produce any toxic changes confirming the safety of the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) in the vital organs.

Conclusion

This study showed that the administration of the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) to Wistar rats was not toxic in any of the tested doses. The Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) did not have a direct impact on the liver and kidney functions as corroborated by results from hematological and blood chemistry analysis on treated rats. Also, the Siddha preparation Uppu chenduram-II at a dose of (200,400 and 600 mg/kg) did not bring about any change in food intake, water consumption or body weight and produced no evident histopathological damage in the rat organs, regardless of gender. Furthermore, the results obtained from both acute and subchronic toxicity studies of Siddha preparation Uppu chenduram-II could thus give insight to its safety in humans.

REFERENCES

1. World Health Organization. Traditional Medicine Strategy 2002–2005. Geneva: World Health Organization; 2002.Available from: http://www.wpro.who.int/healthtechnology/book_who_traditional_medicine_strategy_2002_2005.pdf
2. Taylor JLS, Rabe T, McGaw LJ, Jäger AK, van Staden J. Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 2001;34:23–37.
3. Bashar S, Omar S. Reco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues. 2011.
4. World Health Organization. Traditional medicine. Afr Health Monit. 2003;4(1).
5. Argw TM. Approaches towards evaluation of medicinal plants prior to clinical trials. Found Med Res. 2006;143.
6. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian systems of medicines. J Ethnopharmacol. 2006 Jan 3;103(1):25–35.
7. Patil SB, Patil MS, Chittam KP, Wagh RD. A review on Ayurveda and Siddha: Indian systems of medicine. Pharma Sci Monit. 2014 Apr 1;5(2).
8. Thirumalaivelu B, Chandran S, Uma KS. A review on Siddha formulation Uppu Chenduram for anti-H. pylori and gastroprotective action. Afr J Biomed Res. 2024;27:4179–83. doi:10.53555/AJBR.v27i3S.3132
9. Muthaliyar KNK, Uthamarayan KC. Siddha Vaidya Thirattu. 1st ed. Vol 1. Chennai: Department of Indian Medicine and Homeopathy; 2009.
10. Klaassen CS. Principle of toxicology and treatment of poisoning. In: Parker BK, Blumenthal D, Buxton L, editors. Goodman & Gilman's Manual of Pharmacology & Therapeutics. New York: McGraw Hill; 2008. p. 1115–9.
11. Monosson E. Toxicity testing methods. In: Encyclopedia of Earth [Internet]. [updated 2013]. Available from: http://www.eoearth.org/view/article/1566.73/
12. Organisation for Economic Co-operation and Development (OECD). Guideline for Testing of Chemicals. Guidance No. 425. 2001.
13. Abotsi WK, Ainooson GK, Gyasi EB, Abotsi WKM. Acute and subacute toxicity studies of the ethanolic extract of the aerial parts of Hilleria latifolia (Lam.) H. Walt. (Phytolaccaceae) in rodents. West Afr J Pharm. 2011;22:27–35.
14. Bariweni MW, Yibala OI, Ozolua RI. Toxicological studies on the aqueous leaf extract of Pavetta crassipes (K. Schum) in rodents. J Pharm Pharmacogn Res. 2018;6(1):1–16.
15. Michael B, Yano BL, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol. 2007;35(5):742–50.
16. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague Dawley rats. Toxicol. 2002;179(3):183–96.

17. Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, et al. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicol Pathol. 2007;35(5):751–5.
18. Raina P, Chandrasekaran CV, Deepak M, Aggarwal A, Ruchika KG. Evaluation of subacute toxicity of methanolic/aqueous preparation of aerial parts of Ocimum sanctum in Wistar rats: clinical, hematological, biochemical and histopathological studies. J Ethnopharmacol. 2015;175:509–17.
19. Cajuday LA, Pocsidio GL. Effects of Moringa oleifera Lam. (Moringaceae) on the reproduction of male mice (Mus musculus). J Med Plants Res. 2010;4(12):1115–21.
20. Ezeja MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. J Ethnopharmacol. 2014;151(3):1155–64.
21. Hoffbrand AV, Pettit JE. Hematological parameters. In: Essentials of Haematology. 4th ed. New Jersey: Blackwell Science; 2000.
22. Jaganathan R, Ravinayagam V, Panchanadham S, Palanivelu S. Toxicological, biochemical and histopathological evaluation of Tridham, a Siddha medicine in Wistar albino rats. J Biochem Technol. 2012 May 1;4(1).
23. Gowri R, et al. World J Pharm Res. 2019;8(2):1455. Available from: www.wjpr.net
24. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. 2000;32(1):56– 67.
25. Cavanaugh BM. Nurse's Manual of Laboratory and Diagnostic Tests. Philadelphia: FA Davis Company; 2003.
26. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. Science. 2013;342(6155):1242454.
27. Ganong WF. Review of Medical Physiology. 20th ed. New York: Lange Medical Books, McGraw-Hill; 2001.
28. Bariweni MW, Yibala OI, Ozolua RI. Toxicological studies on the aqueous leaf extract of Pavetta crassipes (K. Schum) in rodents. J Pharm Pharmacogn Res. 2018;6(1):1–16.
29. Adeoye B, Oyedapo OO. Toxicity of Erythrophleum guineense stem-bark: role of alkaloid fraction. Afr J Tradit Complement Altern Med. 2004;1:45–54.
30. Brautbar N, Williams IJ. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int J Hyg Environ Health. 2002;25(6):479–91.
31. Akanji MA, Yakubu MT, Kazeem MI. Hypolipidemic and toxicological potential of aqueous extract of Rauwolfia vomitoria Afzel root in Wistar rats. J Med Sci. 2013;13:253–60.
32. Friedman L, Martin P, Muñoz SJ. Liver function tests and the objective evaluation of the patient with liver disease. In: Zakim D, Boyer TD, editors. Hepatology: A Textbook of Liver Disease. Philadelphia: WB Saunders Co.; 1996. p. 791–833.
33. Tietz NW, Prude EL, Siggaard-Andersen O. Tietz Textbook of Clinical Chemistry. London: WB Saunders; 1994.
34. Guyton AC. Metabolism and temperature regulation. Textbook of medical physiology. 1981:855922.
35. Lum G, Leal-Khouri S. Significance of low serum urea nitrogen concentrations. J Clin Chem. 1989;35(4):639–40