

Preclinical Safety Profiling of Nava Uppu: An Integrated Oral Toxicity Evaluation in Wistar Rats

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ABSTRACT

Nava Uppu (NU) is a traditional Siddha formulation used for various therapeutic purposes. The present study was undertaken to evaluate the acute and sub-acute oral toxicity of NU in female Wistar rats. Acute toxicity was assessed according to OECD Guideline 423 following oral administration of NU at 2000 mg/kg body weight, with animals monitored for mortality, behavioural changes, and clinical signs for 14 days. A 28-day repeated-dose oral toxicity study was conducted according to OECD Guideline 407 using doses of 5, 50, 300, and 2000 mg/kg body weight. General health, food and water intake, body weight, haematological and biochemical parameters, organ weights, electrolytes, and histopathological changes were evaluated. No mortality or treatment-related abnormal behavioural or clinical signs were observed during the acute toxicity study. In the 28-day study, no significant treatment-related changes were observed in haematological parameters, biochemical parameters, electrolyte levels, or relative organ weights. Histopathological examination of the liver and kidneys did not reveal major pathological alterations. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, with $p < 0.05$ considered significant. Overall, the findings suggest that NU did not produce significant acute or repeated-dose toxicity under the experimental conditions and demonstrated preliminary safety and tolerability in Wistar rats.

Keywords: Nava Uppu, Siddha formulation, acute toxicity, sub-acute toxicity, Wistar rats, OECD 423, OECD 407.

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INTRODUCTION:

In south India, siddha medicine is among the oldest traditional medical systems. To maintain health and treat illness, it is predicted on the idea of preserving the balance of the three humors (Vali, Azhal, Iyyam). To improve the therapeutic efficacy and safety of herbal, mineral and Herbo-mineral formulations, siddha treatments employ well-defined pharmaceutical methods. The siddha Vaidhiya Thirattu describes the traditional Herbo-mineral formulation known as Navauppu Mezhu (NUM). It is made by processing 9 types of salts (Navauppu) with herbal substances to create a composition that

resembles wax consistency (Mezhugu). Navauppu Mezhu is recommended for the administration of Soolai, Vali Gunmam, Kiranthi, Thamar vatham and Soothaga Vaayu, abdominal pain, gastrointestinal disturbances, inflammatory disorders and derangements of Vali humor according to the classical text (1). Harbo- mineral formulations have been used therapeutically for a long time. But their quality, purity, safety and consistency still need scientific validation. For these formulations to be used safely for medicinal purposes and to ensure batch-to- batch homogeneity, standardization utilizing contemporary analytical techniques is essential (2). Thus, the goal of current study was to standardize

Navauppu Mezhu using physicochemical analysis, microbiological limit testing, heavy metal estimate, HPTLC fingerprint profiling, organoleptic evaluation, pesticide residue analysis and aflatoxin analysis (3). The present studies provide scientific evidence on the acute and sub-acute safety profile of the formulation, establishing its tolerability and potential toxicological effects and supporting its further pharmacological and therapeutic evaluation.

MATERIALS AND METHODS:

Procurement of the study drug:

The investigational drug was procured from a GMP – complaint pharmaceutical company.

Table. No: 1 Ingredients of Navauppu Mezhu (NUM):

S.no	Name of the drug	Scientific name	Quantity
1	Kariuppu	<i>Sodium chloride</i>	¼ Palam (8.75g)
2	Inthuppu	<i>Sodium chloride impure</i>	¼ Palam (8.75g)
3	Valayaluppu	<i>Potassium nitrate impure</i>	¼ Palam (8.75g)
4	Pottiluppu	<i>Potassium nitrate</i>	¼ Palam (8.75g)
5	Amuriuppu	<i>Ammonium chloride</i>	¼ Palam (8.75g)
6	Mooghiluppu	<i>Bamboo salt</i>	¼ Palam (8.75g)
7	Kalluppu	<i>Sodium chloride</i>	¼ Palam (8.75g)
8	Paaraiuppu	<i>Rock salt</i>	¼ Palam (8.75g)
9	Pooneeru	<i>Sodium carbonate</i>	¼ Palam (8.75g)
10	Veeram	<i>Hydrargyrum Perchloride</i>	1 Palam (35 g)

11	Karpooram	<i>Camphor</i>	½ Palam (17.5g)
12	*Thirikadughu (Chukku, Milaghu, Thippili)	<i>Zingiber officinale</i> <i>Piper nigrum</i> <i>Piper longum</i>	3 Varagan (12.6g)
13	Nervalam	<i>Croton tiglium</i>	¼ Palam (8.75 g)
14	Thummati saaru	<i>Citrullus colocynthis</i>	Required quantity
15	Aavin paal	<i>Cow's milk</i>	Required quantity
16	Thutthi ver	<i>Abutilon indicum</i>	Required quantity
17	*Thiripalai kudineer (Kadukkai, Nellikkai, Thandrikkai)	<i>Terminalia chebula</i> <i>Phyllanthus emblica</i> <i>Terminalia bellirica</i>	Required quantity
18	Thaai Pal	<i>Breast milk</i>	Required quantity

Preparation of Navauppu Mezhu:

All mineral and herbal ingredients were purified following the procedures described in the Siddha literature. Equal quantities (4 Palam each) of Kariuppu, Inthuppu, Valayal Uppu, Pottiluppu, Amuri Uppu, Mooghiluppu, Kalluppu, Paarai Uppu, and Pooneeru were placed in a clean vessel. A mixture of Thummati juice and cow's milk (1 L) was then added, and the contents were stirred thoroughly using the root of Thutthi. After allowing the mixture to settle, the sediment was discarded, and the clear supernatant (Thelivu) was filtered. The filtrate was heated continuously until it attained an in kulambu (thick paste-like) consistency and was further dried to obtain a white-coloured product. The obtained, white-coloured product was transferred to a stone mortar (Kalvam). Finely powdered Veeram, Karpooram, Thirikadughu, and Valam, in the prescribed quantities, were added to the mortar and mixed thoroughly. Thiripalai Kudineer was then added

gradually in the required quantity, and the mixture was triturated continuously for one Samam (3 hours). Subsequently, breast milk was added in the required quantity, and trituration was continued for another Samam (3 hours) until the formulation attained a smooth Mezhugu (wax-like) consistency. The prepared Mezhugu was finally stored in a Kombu Simizh (horn like container) for preservation.

ADMINISTRATION OF THE DRUG:

Table. No: 2 Drug profile

Form of the medicine	Mezhugu
Route of administration	Internal
Dosage	Milaghu alavu (56 mg) (4)
Adjuvant	Karuppati (Palm jaggery)
Duration	¼ Mandalam
Indication	Soolai, Vali Gunmam, Kiranthi, Thamar vatham, Soothaga Vayu.
Dietary regimen (Pathiyam)	Warm water with cooked rice, Roasted salt, cow's milk, buttermilk, sirukeerai (tender greens), murungai pinju (tender drumstick pods), brinjal, green gram, pigeon pea, chicken, quail.

ANIMALS

The present study was conducted in the **Animal House of Arulmigu Kalasalingam College of Pharmacy** after obtaining approval from the **Institutional Animal Ethics Committee (IAEC)** (Approval No.: AKCP/IAEC/46/20-21). A total of **15 female Wistar rats**, approximately **6 weeks old** and weighing **100–150 g**, were used for the study. The animals were housed in clean metabolic cages containing sawdust bedding, with a maximum of three animals of the same sex per cage. The animals were acclimatized to the laboratory environment for **20 days** prior to the commencement of the experiment. A **12-hour light/dark cycle** was maintained throughout the study. Standard pelleted rat feed and drinking water were provided **ad libitum**. During the acclimatization period, the general health condition of the animals was monitored, and the animals were

examined by a veterinary surgeon to ensure that they were healthy and suitable for inclusion in the study.

ACUTE TOXICITY STUDIES:

Six female Wistar rats were chosen at random and split into two groups of three each. Group I served as the control, while Group II was the test, receiving 2000 mg/kg (p. o.) of Nava Uppu solution orally. The animals had free access to water while fasting for the entire night. Every animal was closely observed for any unusual clinical or behavioural abnormalities. The onset, development, and cessation of these clinical signs, if any, were recorded for about 1, 3, and 4 hours after the dose on the day of dosing and each day thereafter for 14 days. From the cage side, changes in the skin, fur, eyes, and mucous membranes were seen, along with the presence of fluids and excretions. Autonomic activity included lacrimation, piloerection, pupil size, and abnormal breathing patterns. Additional behaviours included strange behaviours like self-mutilation and reverse walking, clonic or tonic movements, stereotypes such as excessive grooming and repetitive circling, and alterations in posture, stride, and handling reaction. On the fourteenth day, sensory sensitivity to a variety of stimuli, including auditory, visual, and proprioceptive stimuli, was evaluated. While auditory stimuli responses were evaluated by clicking a sound from about 30 cm away, visual stimulus reactions were captured by shining a pen light in the rats' eyes and placing a blunt object near their eyes. To measure the animal's reaction to proprioceptive stimulation, the anterior/dorsal surface of its paw was pressed against the edge of the table. Both the drug treatment dose group and the control group's animals reacted normally to these three tasks. For 14 days following the initial dose, each animal's mortality and morbidity were assessed twice a day (morning and afternoon) and once daily at about 1, 3, and 4 hours after the dose. Both the drug treatment dose group and the control group's animals reacted normally to these three tasks. For 14 days following the initial dose, each animal's mortality and morbidity were assessed twice a day (morning and afternoon) and once daily at about 1, 3, and 4 hours after the dose. (5)

SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY

The OECD 407 Method-Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents) was used to guide the sub-acute oral toxicity of Nava Uppu tablet solution. The animals were split into five groups at random; the control group was given distilled water, while the other four groups received different doses of drugs (5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg). Over the course of the 28-day treatment, the animals' general health, food and water intake, and clinical signs of toxicity were monitored, while changes in body weight were noted on days 1, 7, 14, 21, and 28 of the experiment. All the animals underwent an overnight fast prior to blood collection at the conclusion of the trial. Under ether anaesthesia, blood samples were taken from

each group via the retroorbital sinus and placed in two different types of tubes: one with EDTA and the other without any additions. The anticoagulated blood (tube containing EDTA) was examined for haematological parameters right away. To extract the serum for biochemical examination, the second tube was centrifuged for ten minutes at 4°C and 3000 rpm. Furthermore, moist slices of the liver and kidneys were evaluated histopathologically after being dissected and weighed.

TERMINAL STUDIES

Sacrifice and macroscopic examination

Ether inhalation was used to kill eighteen Wistar rats after four weeks of treatment. Every animal underwent a comprehensive autopsy, which included both an in situ and post-evisceration inspection of the body's exterior, all orifices, and the contents of the cranial, thoracic, and abdominal cavities. Because there were more animals than could be killed in a single day, the autopsies at the end of the treatment period were spread across three days.

Organ weights:

Following the macroscopic analysis, the weight of the brain, heart, spleen, kidneys, testes, liver, lungs, pancreas, and stomach was determined after the superficial fat was removed.

Haematological analysis:

An automated Bayer Haematology analyser and established protocols were used to evaluate blood samples. Red blood cell count (RBC), white blood cell count (WBC), platelet count, haemoglobin (Hb), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean red cell volume (MCV), mean cell haemoglobin (MCH), mean platelet volume (MPV), neutrophils, eosinophils, basophils, lymphocytes, and monocytes are among the parameters assessed using a haematological analyser. (7)

Biochemical analysis:

The serum was obtained by centrifuging dry tubes containing collected blood at 3000 rpm for 15 minutes at 250C. The serum was then stored at -20 °C until biochemical parameters (serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, and creatinine (Coral clinical system) were measured using an Erba Chem 5 semi-auto analyzer). (8)

Histopathological evaluation:

Histopathological Examination of the Liver and Kidney Tissues. Macroscopic examination of vital organs was carried out soon after sacrifice. Liver and kidneys were surgically removed, weighed (absolute organ weight) on an analytical balance, and immediately forwarded to the histopathological processing. The relative organ weight (ROW) of each animal was then calculated as follows: relative organ weight $\delta \% P = \delta \text{ organ weight/body weight } P \times 100$. (9) After being fixed in a 10% buffered formalin

solution, the liver and kidney fragments were placed in paraffin wax, cut into 3–4 μm slices, and stained with hematoxylin and eosin. After that, the histological sections were photographed using an Infinity 1 camera microscope with $\times 40$ magnification and seen under optical microscopy (Optical Microscopes, Italy).

The observation of tissue integrity and the search for damage such as degeneration, necrosis, apoptosis, and leukocyte infiltration—all of which may be indicators of toxicity—are the components of histopathological investigation. (10)

Statistical analysis:

The statistical analysis will be carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean $\pm$ standard error. A statistical comparison was carried out using Dennett's test for the control and treatment group. P-values less than 0.05 were set as the level of significance. (6)

RESULTS:

TOXICITY STUDIES

Effect of acute Toxicity Study (14 Days) of NU

Table no –3 Physical and behavioral examinations.

Grou p no.	Dose(mg/k g)	Observati on sign	No. of animal affecte d.
Grou p-I	5mg/kg	Normal	0 of 3
Grou p-II	50mg/kg	Normal	0 of 3
Grou p-III	300mg/kg	Normal	0 of 3
Grou p-IV	2000mg/kg	Normal	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=3); $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, calculated by comparing treated groups with control groups. Data obtained in this study indicated $^{ns}p > 0.05$ no significant changes the physical and behavioral signs of any toxicity due to administration of NU at the doses of 5mg/kg, 50mg/kg/kg/kg, 300mg/kg and 2000mg/kg to rats.

Table no-4 Home cage activity

Preclinical Safety Profiling of Nava Uppu: An Integrated Oral Toxicity Evaluation in Wistar Rats

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	2000mg/kg (G-IV)
		Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3
Respiration	Normal	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3
Palpebral closure	Normal	3	3	3	3
ApproTCh response	Normal	3	3	3	3
Touch response	Normal	3	3	3	3
Pinna reflex	Normal	3	3	3	3
Tail pinch response	Normal	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $nsp > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, calculated by comparing treated groups with control groups. Data obtained in this study indicated $nsp > 0.05$ no significance changes in Home cage activity, signs of any toxicity due to administration of *NU* at the doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg to rats.

Table no-5 Hand held observation

Functional and Behavioural observation	Observation	Control	5mg/kg (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	2000mg/kg (G-IV)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3
Handling	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3

Salivation	Normal	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $nsp > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, calculated by comparing treated groups with control groups.

Data obtained in this study indicated $nsp > 0.05$ no significance changes in hand held observation and signs of any toxicity due to administration of *NU* at the doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg to rats.

Table no-6 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	2000(mg/kg)	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $nsp > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, calculated by comparing treated groups with control group. From acute toxicity study it was observed $nsp > 0.05$ that the administration of *NU* at a dose of 2000 mg/kg to the rats does not produce drug-related toxicity and mortality. So, No-Observed-Adverse-Effect- Level (NOAEL) at *NU* is 2000 mg/kg.

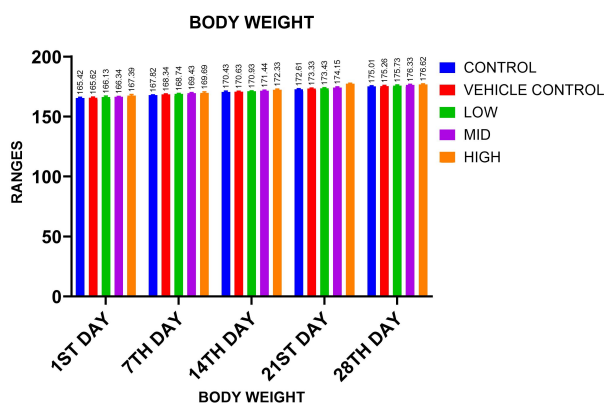
SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF *NU*

Table :7 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *NU* ON BODY WEIGHT IN GRAM

Preclinical Safety Profiling of Nava Uppu: An Integrated Oral Toxicity Evaluation in Wistar Rats

GR OUP	NOR MAL CON TROL	VEHI CLE CON TROL	LOW	MID	HIGH
1 st day	165.42 ±0.64	165.62 ±0.64	166.13 ±0.86	166.34 ±0.36	167.39 ±0.97
7 th day	167.82 ±0.23	168.34 ±0.55	168.74 ±0.56	169.43 ±0.56	169.69 ±0.86
14 th day	170.43 ±0.75	170.63 ±0.45	170.93 ±0.44	171.44 ±0.54	172.33 ±0.54
21 st day	172.61 ±0.63	173.33 ±0.34	173.43 ±0.56	174.15 ±0.57	174.27 ±0.45
28 th day	175.01 ±0.63 *	175.26 ±0.64 *	175.73 ±0.55 *	176.33 ±0.45 *	176.62 ±0.64 *

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control groups.



The effect of NU was observed, on the body weight changes, significantly increase (*p<0.05) in body weight in all the treated animals were observed. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

EFFECT OF SUB-ACUTE DOSE (28 DAYS) NU
Table :8 NU ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP	CONT ROL	VEHI CLE CON TROL	LOW	MID	HIG H
HEART	2.51±0. 44	2.78±0. 53	2.93± 0.44	3.18± 0.33	3.38± 0.64

LIVER		4.18±0. 22	4.28±0. 34	4.46± 0.44	4.60± 0.60	4.83± 0.74
LUNGS		1.16±0. 33	1.23±0. 42	1.38± 0.52	1.45± 0.45	1.51± 0.22
KID NEY	L	0.53±0. 32	0.62±0. 44	0.70± 0.53	0.83± 0.62	0.92± 0.75
	R	0.63±0. 44	0.71±0. 49	0.84± 0.56	0.98± 0.59	1.10± 0.64

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The effects of NU on kidney, heart, liver and lungs of the rats were recorded. Not significant p>0.05 changes in the weights of various organs of the animals occurred with higher doses of the extract but macroscopic examinations visualized no changes in color of the organs of the treated animals compared with the control group.

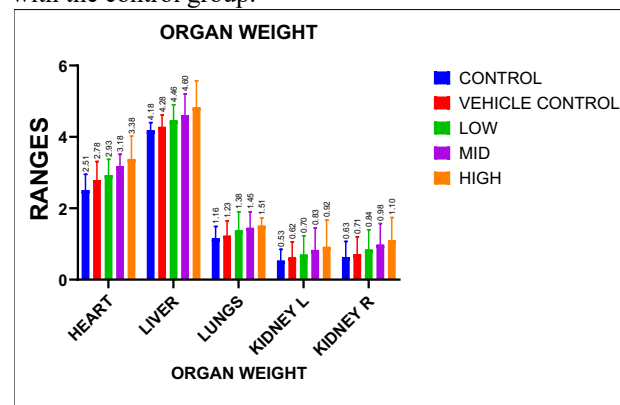


Table no:9 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF NU ON HAEMATOLOGICAL PARAMETERS

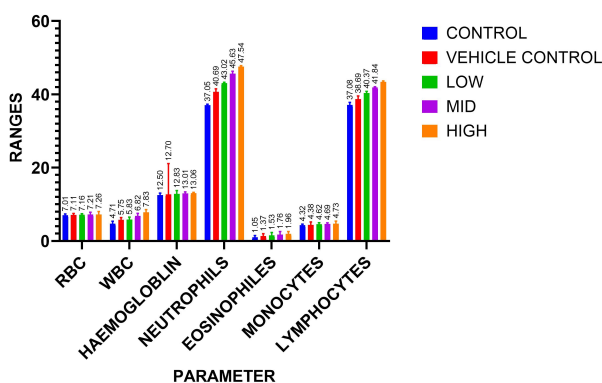
Dru g trea tme nt	RB C 10 ¹² /lite r	WB C 10 ⁹ / lite r	Hae mogl obin gm /liter	Differential count %			
				Neut roph ils	Eosi nop hils	Mo noc yte	Lym phoc yte
Co ntr ol	7.0 1±0 .39	4.7 1±0 .70	12.5± 0.57	37.0 5±0. 28	1.05 ±0.4 6	4.3 2±0 .29	67.0 8±0. 74
Veh	7.1	5.7	12.7±	40.6	1.37	4.3	68.6

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icle Co ntr ol	1±0 .42	5±0 .64	8.43	9±0. 82	±0.6 2	8. ±0. 82	9±0. 86
LO W	7.1 6±0 .28	5.8 3±0 .71	12.83 ±0.93	43.0 2±0. 28	1.53 ±0.7 9	4.6 2±0 .39	60.3 7±0. 46
MI D	7.2 1±0 .64	6.8 2±0 .72	13.01 ±0.39	45.6 3±0. 68	1.76 ±0.8 2	4.6 9±0 .29	71.8 4±0. 23
HI GH	7.2 6±0 .80	7.8 3±0 .75	13.06 ±0.20	47.5 4±0. 29	1.96 ±0.6 2	4.7 3±0 .72	73.3 7±0. 28

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control groups.

HAEMATOLOGICAL PARAMETER



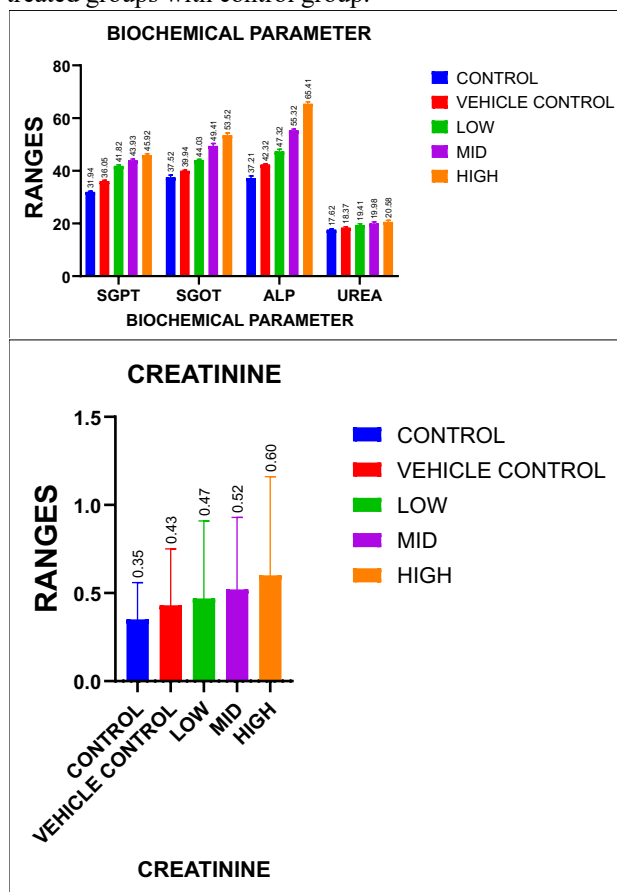
The effects of NU were observed for its effect on hematological parameters in experimental rats. Final study, not significant (p<0.05) in the haemoglobin,RBC values are increased after treated groups. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

Table :10 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF NU ON BIOCHEMICAL PARAMETER

Drug Treatment	SGPT (U/L)	SGOT(U/L)	ALP(U/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	31.94±0.29	37.52±0.83	37.21±0.83	17.62±0.29	0.31± 0.55
Vehicle Control	36.05±0.33	39.94±0.29	42.32±0.29	18.37±0.39	0.38±0.65
LOW	41.82±0.37	44.03±0.29	47.32±0.82	19.41±0.47	0.37±0.56
MID	43.93±0.47	49.41±0.94	55.32±0.48	19.98±0.58	0.42±0.56
HIGH	45.92±0.53	53.52±0.84	65.41±0.72	20.58±0.63	0.47±0.47

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05,

p<0.01, *p<0.001, calculated by comparing treated groups with control group.



The SGPT, SGOT, ALP, UREA and Creatinine values was compared in Group I and other groups II, III, IV. The biochemical Parameters in experimental rat. not significant (p<0.05) in SGPT, SGOT, ALP, urea and Creatinine values. The values are expressed as mean ± S.E.M. n=6.

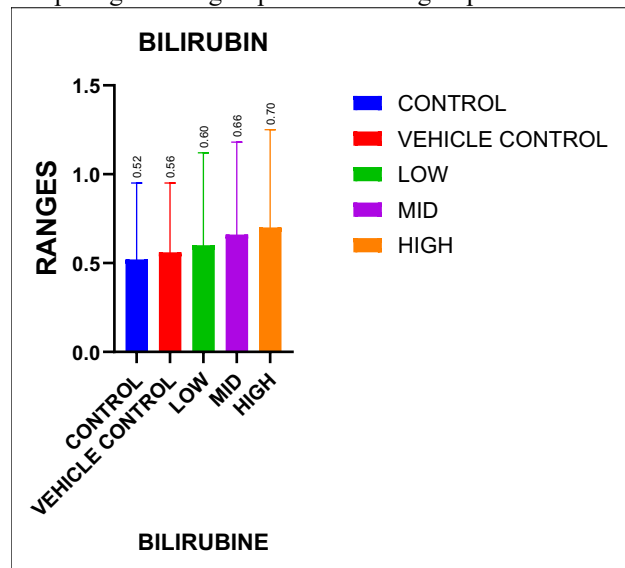
Table : 11 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF NU BIOCHEMICAL PARAMETERS

GR	CO	VE	L	M	HI
OU	NT	HIC	O	ED	G
P	RO	LE	W	IU	H
	L	CO		M	
		NT			
		RO			
		L			
TO			0.3	0.3	0.4
TA	0.23		3±	7±0	5±
L	±0.4	0.27	0.6	.78	0.5
BIL	9	±0.3	5		9

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IRU		9			
BIN					
(mg/dl)					

Values are expressed as mean $\pm$ SEM
Statistical significance (p) calculated by one-way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.



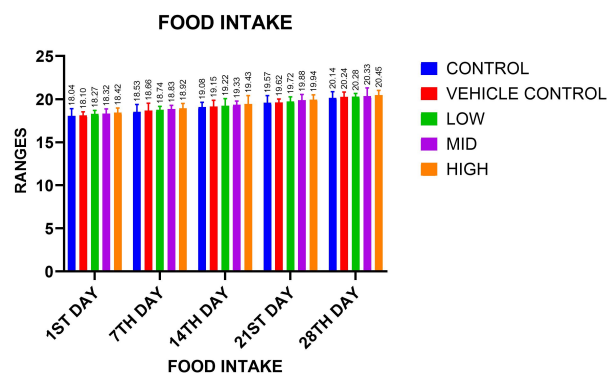
The Bilirubin values experimental rat. Final study, not significant (p<0.05) in the bilirubin values are. The values are expressed as mean $\pm$ S.E.M. n=6 was compared in Group I and other groups II, III, IV, and V.

Table:12EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF ON FOOD INTAKE IN GRAM

GR OUP	CONT ROL	VEHI CLE CONT ROL	LOW	MEDI UM	HIGH
1 st DAY	18.04 $\pm$ 0.86	18.10 $\pm$ 0.42	18.27 $\pm$ 0.42	18.32 $\pm$ 0.54	18.42 $\pm$ 0.55
7 th DAY	18.53 $\pm$ 0.85	18.66 $\pm$ 0.86	18.74 $\pm$ 0.42	18.83 $\pm$ 0.45	18.92 $\pm$ 0.57
14 th DAY	19.08 $\pm$ 0.55	19.15 $\pm$ 0.72	19.22 $\pm$ 0.84	19.33 $\pm$ 0.44	19.43 $\pm$ 0.97
21 st DAY	19.57 $\pm$ 0.83	19.62 $\pm$ 0.39	19.72 $\pm$ 0.54	19.88 $\pm$ 0.66	19.94 $\pm$ 0.56
28 th	20.14 $\pm$	20.24 $\pm$	20.28 $\pm$	20.33 $\pm$	20.45 $\pm$

DAY	0.73*	0.58*	0.38*	0.97*	0.56*
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Values are expressed as mean $\pm$ SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group



The Food intake values were compared in Group I to other groups II, III, IV, and V. in experimental rat. Final study, significantly increase in the Food intake values. The values are expressed as mean $\pm$ S.E.M. n=6.

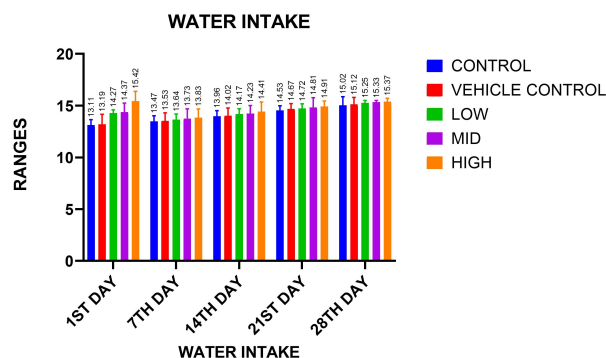
Table:13. Effect of Sub- Acute Dose (28 Days) Of NU On Water Intake in ml

G R O U P	CO NT RO L	VE HIC LE CO NT RO L	LO W	MI DU M	HI GH
1 st D A Y	13.1 1 $\pm$ 0. 53	13.1 9 $\pm$ 0. 97	14. 27 $\pm$ 0.3 3	14. 37 $\pm$ 0.8 7	15. 42 $\pm$ 0.9 6
7 th D A Y	13.4 7 $\pm$ 0. 55	13.5 3 $\pm$ 0. 77	13. 64 $\pm$ 0.5 4	13. 73 $\pm$ 0.9 6	13. 83 $\pm$ 0.8 5
14 th D A Y	13.9 6 $\pm$ 0. 55	14.0 2 $\pm$ 0. 75	14. 17 $\pm$ 0.5 3	14. 23 $\pm$ 0.7 8	14. 41 $\pm$ 0.9 4
21 st D A Y	14.5 3 $\pm$ 0. 45	14.6 7 $\pm$ 0 53	14. 72 $\pm$ 0.4 5	14. 81 $\pm$ 0.9 6	14. 91 $\pm$ 0.5 4

Preclinical Safety Profiling of Nava Uppu: An Integrated Oral Toxicity Evaluation in Wistar Rats

28 ^t 9h			15.	15.	15.
D	15.0	15.1	25±	33±	37±
A	2±0.	2±0.	0.2	0.1	0.3
Y	85	68	5	7	5

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

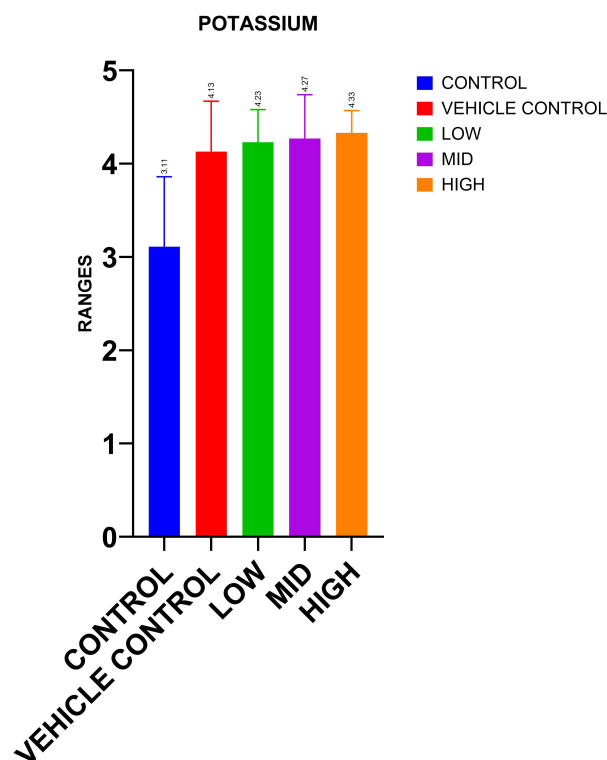
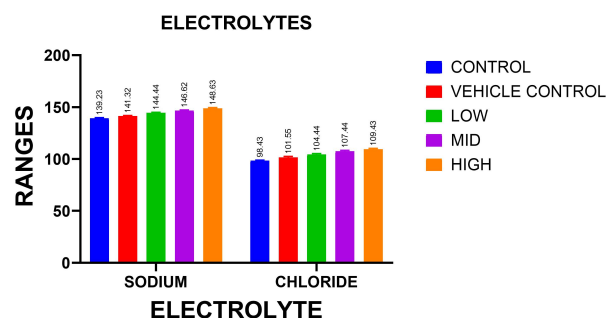


The water intake values were compared in Group I and other groups II, III, IV, and V. in experimental rat. Final study, significantly increase in the water intake. The values are expressed as mean ± S.E.M. n=6

Table: 14 EFFECT OF SUB-ACUTE DOSES (28 DAY) OF NU ON ELECTROLYTES: -

GROUP	CONTRO L	VEHICLE CONTRO L	LOW (300mg/kg)	MEDIUM (1000mg/kg)	HIGH (2000mg/kg)
Sodium (mmol/L)	139.23±0.6	141.32±0.5	144.44±0.5	146.62±0.86	148.63±0.86
chloride(mmol/L)	98.43±0.55	101.54±0.9	104.44±0.8	107.44±0.96	109.43±0.86
potassium(mmol/L)	3.11±0.75	4.13±0.54	4.23±0.65	4.27±0.47	4.33±0.24

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dennett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group



ELECTROLYTE - POTTASSIUM

There were no significant changes in the Electrolyte level in all the treated animals compared to the control. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV and V

Discussion

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food and water consumed by animals also significantly increases. The results of hematological investigations conducted on day 29th day revealed no significant changes in the hematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by NU. The clinical biochemistry analysis was done to evaluate the possible alterations in hepatic and renal functions not influenced by the test drug. Results of Biochemical investigations conducted on days 29 and recorded in revealed no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT, SGPT, Bilirubin were within the

limits. Group Mean Relative Organ Weights are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be normal comparable with respective control group.

CONCLUSION

Acute and subacute toxicity were carried out in wister albino rats according to OECD guidelines (423) This drug has no acute toxicity as there was no mortality seen.

Sub-acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, functional observation, hematological and biochemical investigations were carried out. There were no significant changes in the biochemical and haematological profile. So, the toxicological study of these test drug, NU establish the safety of the drug for long time administration.

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