ACUTE AND SUB ACUTE DERMAL TOXICITY OF SIDDHA FORMULATION - NUNAPATTAI THAILAM

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***Abstract*- Nunapattaithailam (NPT) is being used to treat various skin ailments including Atopic dermatitis. This study aims to investigate the acute & sub Acute dermal toxicity of NPT following single repeated doses of exposure.**
A group of 24 Adult rats ie. 4 groups (n=6per group) for both acute & sub acute dermal toxicity. The duration for each study is determined to be 14 days for Aute toxicity and 28 days for subacute toxicity.
The rats were Applied with the liquid test substance on the shaved area of dorsal surface of the trunk of the test Animal.
For Acute toxicity the rats in both groups recieved the application of the liquid test substance on the first day of the study, and the rats in subacute toxity were given the liquid test Substance for 28 days.
Throughout the study period all the rats were observed for changes in physical, Appearance & Behavioural pattern observed to All the rats.
Body Weight, Haematology,serum Biochemistry result shows no significant changes in both groups
Thus this study indicates that the tropical application of NPT dose not induce any aute & subacute adverse effects on the skin or systemic toxic reactions in Rats.

***Key Words* :Dermal toxicity, Nunapattai thailam,Haematology,Liver enzymes,Histology.**

INTRODUCTION

Nunapattai thailam has been used in the treatment of various skin ailments as mentioned in siddha classical literature.
Tropical Application of NPT is used in treatment of Atopic dermatitis.
For the treatment Application, evaluation of acute and subacute toxicity effects of the preparation is important, to know whether it causes any adverse drug reaction to make sure of its Safety before it could be administered for therapeutic purposes.
According to OECD guidelines 402, In acute dermal toxicity, adverse effects are produced with a short period of application of the test substance.
Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a test substance. Dose is the amount of test substance applied. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g. mg/kg)
Principle of the test method: The test substance is applied to the skin in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects and deaths are made. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied. Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

PROCEDURE:

Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomised and assigned to the treatment groups. Approximately 24 hours before the test, fur should be removed from the dorsal area of the trunk of the test animals by clipping or shaving. Care must be taken to avoid abrading the skin, which could alter its permeability. Not less than 10 per cent of the body surface area should be clear for the application of the test substance.
The weight of the animal should be considered when deciding on the area to be cleared and on the dimensions of the covering. When testing solids, which may be pulverized if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test substance should be considered. Liquid test substances are generally used undiluted.

Experimental animals:

The adult rats are used. The following weight ranges are suggested to provide animals of a size which facilitates the conduct of the test: rats, 200 to 300 g, animals with healthy, intact skin is used.
At least 6 animals are used at each dose level. They should all be of the same sex. If females are used, they should be nulliparous and non-pregnant. The use of a smaller number of animals may be justified in some cases. Where information is available demonstrating that a sex is markedly more sensitive, animals of this sex should be dosed.

Housing and feeding:

The temperature of the experimental animal room should be 22°C (± 3°) for rodents and the relative humidity is 30-70 per cent. Where the lightings are artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets will be used with an unlimited supply of drinking water

Observation period

The observation period is least by 14 - 28 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and may be extended when considered necessary. The time at which signs of toxicity appear and disappear, their duration and the time of death are important, especially if there is a tendency for deaths to be delayed.

Performance of the test:

The test substance is applied uniformly over an area which is approximately 10 per cent of the total body surface area. The surface area should be covered with as thin and uniform film as possible.

Test substances should be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site should be further covered in suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance

Following application of the test substance, the animals should be observed frequently during the first day and then a careful clinical examination should be made at least once each day. Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals

Cage side observations should include changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The time of death must be recorded

Individual weights of animals should be determined shortly before the test substance is applied, weekly thereafter, and at death; changes in weight should be calculated and recorded when survival exceeds one day. At the end of the test surviving animals are weighed and then sacrificed.

Information on experiment:

Species	Strain	Source	Environmental conditions	Sex	Time of dosing	Time of death	Signs of toxicity
Rat	SD	Mass Biotech Chennai	Normal lab conditions	F	10 a.m	Nil	No

Experiment group assignment in SD rats and mortality:

Groups	Sex	No of animals	Dose	Mortality
Vehicle control	Female Male	06	Twice daily applied over shaved skin	0/6
Nunapattai Thailam (NPT) 100%	Female Male	06	Twice daily applied over shaved skin	0/6

Repeated dose dermal toxicity: 28 days (Test-limit): OECD 410 on Nunapattai Thailam (NPT)

The study was conducted according to the recommendations of the OECD guideline No 410

The test was performed on 12 SD male rats

Group I – Control group

Group II—NPT group

NPT was applied topically, at the dose of 1ml/Kg BW site of about 6 cm square once a day for a period of 28 days for testing animals

Body weight measurements were taken every week. Fur was clipped, carefully to avoid abrading the skin, from the dorsal area of the trunk 24 h prior to the test and repeated at a weekly interval

Observation was done to record the signs of toxicity treated areas were observed for erythema, edema, skin thickening, cracks, scabs and hair growth. At the end of the experiment, the animals were fasted overnight, and for haematologic and biochemical analysis

RESULT

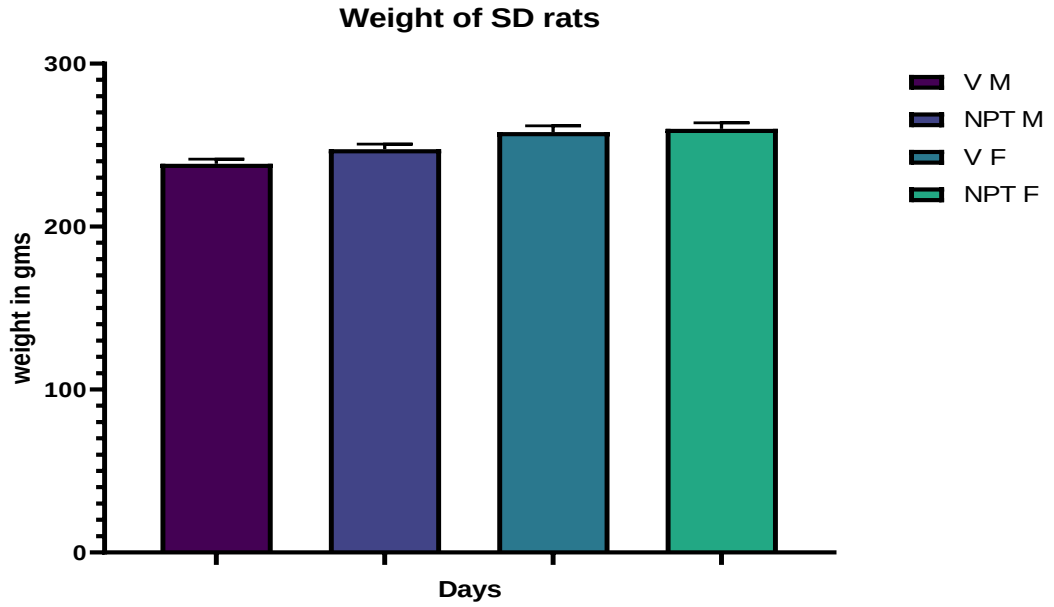
Sub-acute dermal toxicity clinical signs

Sex	Group	Dose (mmol.)	No of animals	Erythema & Eschar	Edema
				Days	Days

				1	7	14	28	1	7	14	28
Male	**Vehicle**	0	06	0	0	0	0	0	0	0	0
	NPT	100%	06	0	0	0	0	0	0	0	0
Female	**Vehicle**	0	06	0	0	0	0	0	0	0	0
	NPT	100%	06	0	0	0	0	0	0	0	0

Body weight of dermal toxicity:

Sex	**Group**	**Dose (mmol.)**	**No of animals**	**Body weight in gms**			
				Day 1	Day7	Day 14	Day 28
Male	**Vehicle**	**0**	**06**	235.2±1.06	237.4±1.07	239.6±1.06	241.8±1.52
	NPT	**100%**	**06**	243.6±1.08	246.6±1.04	248.5±1.12	251.2±1.44
Female	**Vehicle**	**0**	**06**	253.3±1.11	256.7±1.03	259.8±1.10	262.1±1.41
	NPT	**100%**	**06**	256.2±1.10	258.2±1.05	261.6±1.11	264.3±1.71



Behavioural Signs of Toxicity for NPT

Sl	**Group Vehicle control**	**Observation**	**Sl**	**Group NPT group**	**Observation**
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal

5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

SERUM BIOCHEMISTRY

Effect of the acute dermal exposure of NPT on selected parameters

Parameter	**V.Control**	**NPT**
B 8AST(U/L)	202.6±5.78	182.5±6.23
ALT(U/L)	88.2±4.21	85.6±3.01
Albumin (g/dl)	2.58±1.20	2.40±3.73
Globulin (g/dl)	3.96±5.12	4.05±7.20
Total protein(g/dl)	6.78±1.02	6.72±3.06
Creatinine (mg/dl)	0.57±0.04	0.54±0.02

INTERPRETATION

The acute and sub-acute dermal toxicity study results show no mortality and no signs of toxicity.

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