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Acute and Subacute Toxicity Study of *Kadalazhinjil Kudineer* in Swiss Albino Female Rat and Sprague Dawley Rats

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S. Gopika

Villupuram – 605 602, TamilNadu, India.

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ABSTRACT

Siddha medicine is one of the ancient medicine. Lore *Shiva* is the first saint to describe the *Siddha* Medicine to *Nandhi Devar* and he passed on to other saints. It is all about balancing the three senses of humour (*Vaatham*, *Pitham*, *Kabam*) in our body by taking ***Arusuvai Unavu*** (*Inippu*, *Pulippu*, *Uppu*, *Kaippu*, *Karppu*, *Thuvarppu*) in our meals. Mahatma Gandhi also quoted that, **"It is Health that is real wealth and not the pieces of Gold and silver"** Single herb formulations help in identifying its efficacy against the particular disease and the mechanism by which it is acting in our body. Herbs are the safest forms of medicines for children. Tinea infection is common among 5-16yrs of age. In India, the prevalence of Tinea infection is around 1 – 20yrs of age. As per WHO Guidelines, before performing the clinical trial, preclinical studies should be undergone. The present preclinical study aimed to carry on safety and toxicity of *Kadalazhinjil Kudineer*. Acute and Sub-Acute Toxicity studies were carried according to as per OECD Guidelines 423 and 407. Hematological, Biochemical, Histopathological studies were performed for all animals. The study concludes that an oral administration of test drug at both the dose level of 200mg/kg and400mg/kg may be considered as relatively safe, as it did not cause either mortality or produce severe toxicological effects on selected body organs, biochemical indices and haematological and histopathological markers of rats during the sub-acute periods of study.

INTRODUCTION:

THE DERMATOPHYTES (RINGWORM)

These disorders are caused by a group of group of closely related fungi filamentous fungi. They affect the Stratum Corneum, hairs and nails.

Genera responsible for Dermatophyte infections:

- *Trichophyton*
- *Microsporum*
- *Epidermophyte*

***Trichophyton* species:**

They cause lesions of all keratinized tissues including the skin, hair, and nails. They are more virulent than others. They can be acquired from both human and nonhuman sources.

***Microsporum* species:**

They principally invade the hair. They can be acquired from both human and nonhuman sources.

***Epidermophyton* species:**

They invade the intertriginous skin. They are transmitted only by humans.

Types of Dermatophytoses:

- Geophilic - Fungi acquired from the soil.
- Zoophilic - Fungi acquired from the animals.
- Anthrophilic - Fungi acquired from the humans.

Classification of Tinea infections:

➢ Tinea Capitis

- Tinea Pedis
- Tinea Mannum
- Tinea Unguium
- Tinea Barbea
- Tinea Cruris
- Tinea Corporis

TINEA

(Athlete's Foot, Jock Itch, and Ringworm)

Tinea (ringworm) is any of a variety of skin mycoses. Tinea is a very common fungal infection of the skin. Tinea is often called **"Ringworm"** because it is circular, and has a "ring-like" appearance.

Table 1: Classification of Tinea Infections

Sr. No.	Classification of Tinea Infections	Other Name	Causative Organisms
1.	Tinea Capitis	Ring Worm of the Scalp or TineaTonsurans.	*Microsporumcanis, M. Audonii, M. Verucosum, Trichophytontonsurans.*
2.	Tinea Pedis	Ring Worm of the Foot or Athlete's Foot.	*T. rubrum, T. mentagrophytes, Epidermophytonfloccosum.*
3.	Tinea Unguium	Ring Worm of the Nail.	*T. rubrum, T. mentagrophytes.*
4.	Tinea Barbea	Ring Worm of the Beard or Barber's Itch.	*T. mentagrophytes, T. verrucosum.*
5.	Tinea Cruris	Ring Worm of the Groin or Dhobi's Itch or Jocker Itch.	*E. flocossum, T. rubrum, T. mentagrophytes.*
6.	Tinea Corporis	Ring Worm of the body or TineaCircinata.	*T. rubrum, T. verrucosum, M. canis.*

Prevalence of Tinea Infections:

- It is prevalent in tropical countries.
- Tinea infection is common among 5-16yrs of age.
- In India, the prevalence of Tinea infection is around 1 – 20yrs of age[5][6].

Incubation period:

The incubation period differs:

- TineaCorporis has an incubation period of 4 - 10days.
- TineaCapitis has an incubation period of 10–14 days.
- The incubation period of TineaPedis and TineaUnguium is probably weeks but exact limits are unknown.

Public health significance & occurrence:

- Tinea Capitis mainly affects children.
- *Microsporum Canis* is usually contracted from infected Kittens or Puppies.
- The highly contagious *Microsporumaudonii* spreads from person to person.
- Tinea Capitis may extend to Tinea Corporis. It occurs worldwide.
- Tinea Corporis occurs worldwide and relatively frequent. Males are infected more than females. Infection can occur from direct or indirect contact with Skin and Scalp lesions of infected persons or animals.
- Tinea Pedis occurs in children and adults and is spread by using communal facilities such as showers at Swimming Pools. Infection is more frequent and severe in hot weather.
- Tinea Unguium occurs commonly. It is spread by direct contact with Skin or Nail lesions of infected persons or indirectly through contact with contaminated Floors or Showers.

Reservoir:

Reservoirs for Tinea are:

- Tinea Capitis: Humans and Animals including Dogs, Cats, and Cattle.
- Tinea Corporis: Humans, Soil, and Animals including Cattle, Kittens, Puppies, Guinea Pigs, Mice and Horses.
- Tinea Pedis: Humans.
- Tinea Unguium: Humans and rarely Animals or Soil.

Mode of transmission:

- Direct transmission occurs through human to human contact, for example, *Trichophytonrubrum* and *Trichophytonmentagrophytes*.
- The animal-to-human contact also occurs, for example, Microsporum *Canis* and *Trichophytonverrucosum*.
- Tinea can be transmitted indirectly through contaminated soil, for example, *Microsporumgypsum*.

Period of communicability:

The fungus persists on contaminated materials as long as lesions or animal hair harbour viable spores.

Susceptibility & resistance:

- Young children are particularly susceptible to Tinea Capitis (*Microsporum Canis*). All ages are susceptible to infections particularly those caused by *Trichophyton* spp.
- Susceptibility to TineaCorporis is widespread. It is aggravated by friction and excessive perspiration in Axillary and Inguinal regions, and when environmental temperatures and humidity are high.
- Susceptibility is variable for TineaPedis and infection may be unapparent. Repeated attacks are frequent [2][3][4].

Prior to the human trial, the safety of the trial drug has to be ensured [9]. So, a preclinical study is important to determine the safety dose for human trial. The present preclinical study is aimed to evaluate the acute and subacute toxicity of *KadalazhinjilKudineer*. This study provides vital information about the safety and efficacy of *KadalazhinjilKudineer*.

MATERIALS AND METHODS:

Kadalazhinjil Kudineer[1] is a single herbal formulation which consists of *Kadalazhinjil* (*Salaciareticulata*).

Kadalazhinjil is made into a coarse powder. Water is added and boiled to get 1/8 measure of the decoction. Filtered and consumed.

ACUTE ORAL TOXICITY – OECD GUIDELINES - 423

An acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-423. This study was approved by Institutional Animal Ethical Committee (IAEC), C. L. BaidMetha College of Pharmacy, Thoraipakkam, Chennai – 97.

Animal: Healthy Swiss Albino Female Rat weighing 220–240 gm.

Studied carried out at three female Rats under the fasting condition, signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

Introduction:

The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Principle:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing is needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of animal species:

The preferred rodent species is Rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss Albino are used. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within ±20% of the mean weight of the animals.

Housing and feeding conditions:

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hrs light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Observation was done:

Table 2: Observations of animal

Group	**Day**
Body Weight	Normal
Assessments Of Posture	Normal
Signs Of Convulsion Limb Paralysis	Absence Of Sign (-)
Body Tone	Normal
Lacrimation	Absence
Salivation	Absence
Change In Skin Colour	No Significant Colour Change
Piloerection	Normal
Defecation	Normal
Sensitivity Response	Normal
Locomotion	Normal
Muscle Gripness	Normal
Rearing	Mild
Urination	Normal

Dose Mg / kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-

1. Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19 Respiration 20. Mortality.

Table 3: Effect of Test drug on Mortality rate of the study animals on acute toxicity study

TREATMENT	MORTALITY OBSERVED FOR THE DURATION OF 1- 14 DAYS
GROUP I - CONTROL	NIL
GROUP II - TREATMENT	NIL

SUBACUTE TOXICITY STUDIES

28 days Sub Acute Toxicity Studies:

The 28-day repeated oral toxicity study was performed according to OECD guidelines 407 - Repeated Dose 28-Day Oral Toxicity Study in Rodents. Forty young healthy adult Sprague Dawley Rats weighing 100-120 g B.Wt were used for the study. Animals were housed individually in a well ventilated Polypropylene cage. A 12-h light/12-h dark artificial photoperiod was maintained. Room temperature 22°C (±3°C) and relative humidity 50–70% were maintained in the room. Animals had free access to pelleted feed (M/s. Provimi Animal Nutrition India Pvt. Ltd, India) and purified water *ad libitum*.

Animals were acclimatized for a period of 7 days to the laboratory conditions prior to initiation of the experiment and randomized into four groups (10 animals/group; 5/sex) based on stratified body weight method.

Animals received test drug by oral gavage once daily for a period of 28 days.

Observation

- Following test drug administration, experimental animals were observed daily for clinical signs till completion of the experiment.

- **Blood Parameters:** Blood samples were collected through retro-orbital puncture on day 28. Prior to blood collection, the animals were overnight fasted but had free access to water. The following parameters were analyzed in the blood samples.

➢ **Haematology** - Hematocrit (HCT), Hemoglobin (HGB), Total Erythrocyte Count (RBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular volume (MCV), Mean

Corpuscular Hemoglobin Concentration (MCHC), Platelet Count and Total Leucocytes (WBC).

- **Biochemistry**
- Carbohydrate mechanism: Glucose
- Lipid metabolism: Total cholesterol, triglycerides
- Protein metabolism: Total protein, albumin
- Liver function

Hepatocellular: Glutamyl pyruvate aminotransferase (SGPT),

Hepatobiliary: Alkaline phosphatase, γ-glutamyl transferase, total bilirubin, bile acid

- Renal function: Creatinine and urea

- **Necropsy:** All survived animals were sacrificed using CO_2 euthanasia on day 28. The following were the observations carried out during necropsy.

- **Histopathology** - Histopathology examination was performed for below-mentioned organs of animals from control and high dose groups and for the organs from low and mid dose groups that showed evidence of gross abnormalities. Organs such as Skin with Mammary Gland, Lymph Nodes, Eyes, Brain, Trachea, Thyroid, Thymus, Heart, Lungs, Stomach, Small And Large Intestines (with Peyer's Patches), Spleen, Liver, Adrenals, Kidneys, Urinary Bladder, Testes, Epididymis, Male Sex Glands (as whole), Ovaries, Uterus With Cervix, Vagina, Peripheral Nerve, Skeletal Muscle, Bone with Bone Marrow and Spinal Cord of all the animals were collected and fixed in 10% Neutral buffered formalin for 48 hrs, processed for paraffin embedment, sectioned and stained with H&E for histopathological evaluation.

Data analysis

Data were expressed as mean ± SEM. An appropriate statistical analysis was performed to analyze the mean difference between the control and test drug administered groups. P value

≤0.05 was fixed as significance criterion. Statistical analysis was performed in GraphPad Prism 5.0.

Sample No: GopikaLD	**Sample ID: ADC 2743/2015**
Ref.by. : C.L.BAID METHA COLLEGE OF PHARMACY	**Received Date: 11.03.2015**

HAEMOTOLOGY

CBC

WBC : 8,300±0.024 cells/cumm

DIFFERENTIAL COUNT

NEUTROPHILLS : 13±0.421 %

LYMPHOCYTES : 86±0.200 %

EOSINOPHILS : 01±0.320 %

MONOCYTES : 00 %

RBC : 9.09±0.24millions/cumm

HB : 16.6±0.281gms%

PCV : 54.2±0.226 %

MCV : 59.6±0.224fL

MCH : 18.3±662pg

MCHC : 30.6±228 Grams/dl

PLATELET : 7.28±0.112Lakhs/cumm

Sample No: 608LD	**Sample ID: ADC 2743/2015**
Ref.by. : C.L.BAID METHA COLLEGE OF PHARMACY	**Received Date: 11.05.2015**

BIOCHEMISTRY

Blood sugar : 82±0.420mg/dl

BUN : 43.4±0.224 mg/dl

Creatinine : 0.8±0.332mg/dl

SGOT : 76±0.221U/L

SGPT : 62±0.228U/L

ALP : 120±0.521U/L

T.Protein : 8.3±0.222grams/dl

Albumin : 4.3±0.322grams/dl

LIPID PROFILE

T. Cholesterol : 110±0.023mg/dl

Triglycerides : 65±0.004mg/dl

HDL : 23±mg/dl

LDL : 74±0.021mg/dl

VLDL : 13±0.024mg/dl

Ratio 1(T.CHO/HDL): 4.78±0.222

Ratio 2(LDL/HDL) : 3.2±0.224

Sample No: GopikaHD	**Sample ID: ADC 2744/2015**
Ref.by. : C.L.BAID METHA COLLEGE OF PHARMACY	**Received Date: 11.03.2015**

HAEMOTOLOGY

CBC

WBC	:	10,000±0.242cells/cumm

Differential Count

NEUTROPHILLS	:	10±0.521%
LYMPHOCYTES	:	89±0.245 %
EOSINOPHILS	:	01±.004 %
MONOCYTES	:	00 %
RBC	:	8.78±0.420millions/cumm
HB	:	16±0.542gms%
PCV	:	50.3±0.421 %
MCV	:	57.3±0.042fL
MCH	:	18.2±0.268pg
MCHC	:	31.8±0.654 Grams/dl
PLATELET	:	4.8±0.621Lakhs/cumm

Sample No: 608HD	**Sample ID: ADC 2744/2015**
Ref.by. : C.L.BAID METHA COLLEGE OF PHARMACY	**Received Date: 11.05.2015**

BIOCHEMISTRY

Blood sugar	:	89±0.821 mg/dl
BUN	:	50.9±0.468 mg/dl
Creatinine	:	0.9±0.004 mg/dl
SGOT	:	82±0.004 U/L

SGPT : 59±0.024 U/L

ALP : 140±0.224U/L

T.Protein : 8.0±0.221 grams/dl

Albumin : 3.9±0.226grams/dl

LIPID PROFILE

T. Cholesterol : 105±0.864 mg/dl

Triglycerides : 68±0.850mg/dl

HDL : 22±0.046mg/dl

LDL : 69.4±0.064mg/dl

VLDL : 13.6±0.042mg/dl

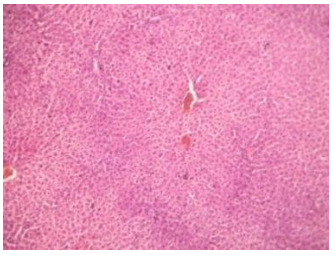
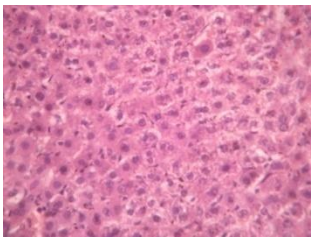
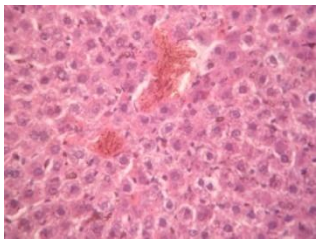
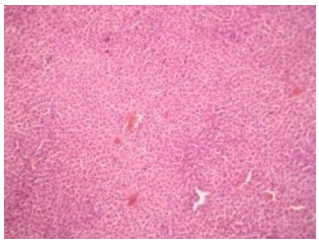
Ratio 1(T.CHO/HDL) : 4.77±0.422

Ratio 2(LDL/HDL) : 3.1±0.112

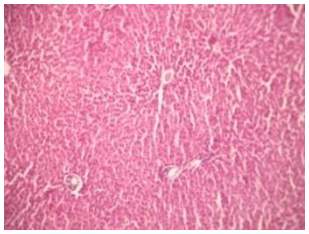
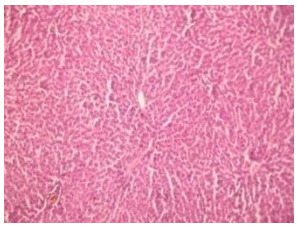
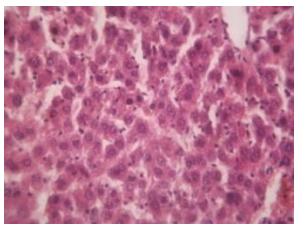
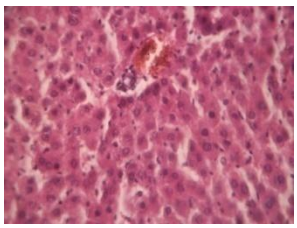
HISTOPATHOLOGICAL STUDY REPORT

SAMPLE: LIVER

GROUP: LOW DOSE GROUP

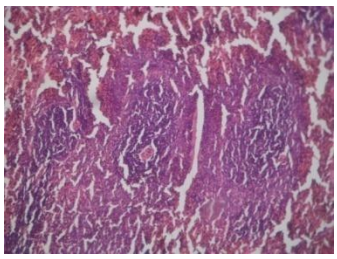
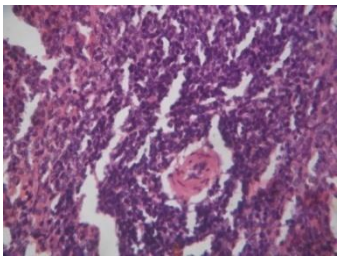
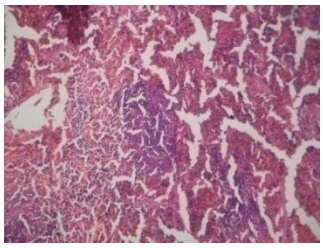
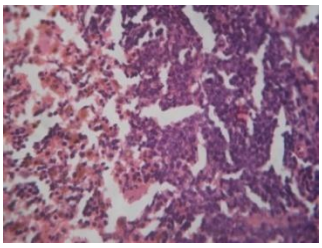





GROUP: HIGH DOSE GROUP

SAMPLE: SPLEEN

GROUP: LOW DOSE GROUP

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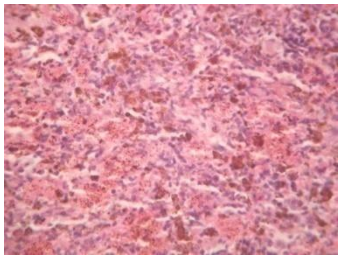
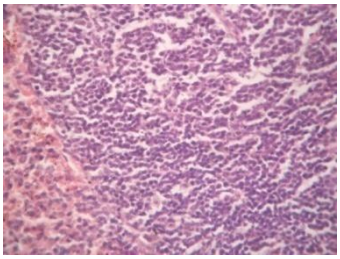
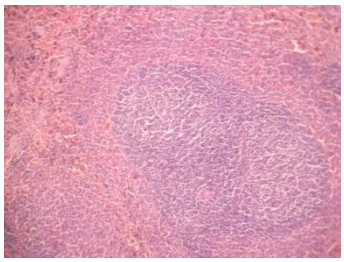
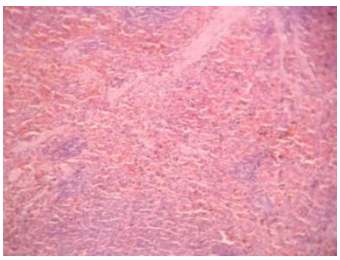
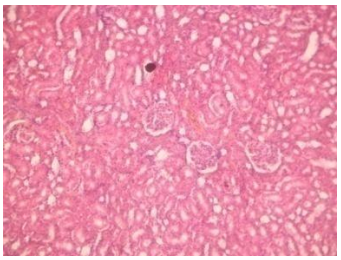
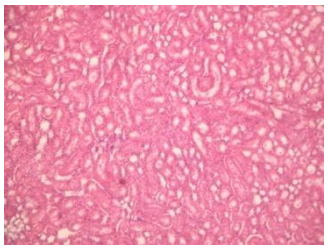
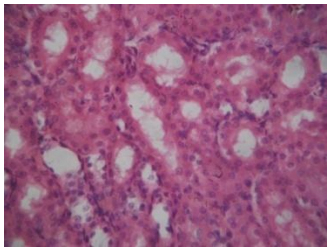
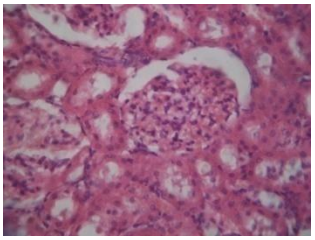
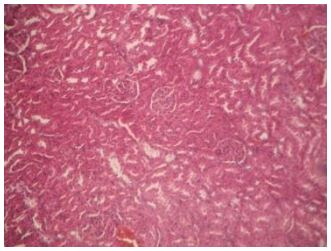
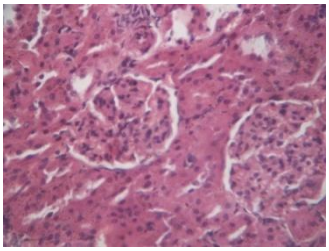
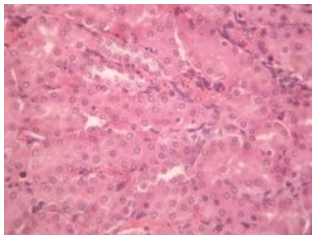
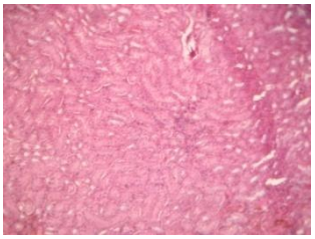





SAMPLE: KIDNEY

GROUP: LOW DOSE GROUP

GROUP: HIGH DOSE GROUP

PATHOLOGIST REPORT

Sample	Observation
Liver	Hepatic Parenchymal lining appears normal An arrangement of Hepatocytes was intact with prominent nuclei stained. Hepatic veins appear normal. No signs of necrosis or cirrhosis. No signs of inflammation in all the three groups.
Spleen	Fibrous capsule of the spleen appears normal. Spleenic sinuses appear normal. No signs of hemorrhage.
Kidney	Lumen of the kidney appears normal in all the three groups Appearance and arrangement of the nephrotic bundle in all the three groups also normal. Renal cortex and medulla appears normal Arrangement of nephrotic bundles appears regular and highly intact

RESULT AND DISCUSSION:

Acute toxicity study

Acute toxicity effect of the test drug was estimated by close observation of animals for about 24 hours after single dose administration of the test drug and it was observed that there are no significant signs of C.N.S related toxicity like convulsion, locomotion, muscle strength and A.N.S related toxicity Like Salivation, Lacrimation etc was observed in treatment group .At the end of the study period, all animals were sacrificed and the organs were isolated and observed for change in structural morphology. There is no significant change in the organ necropsy of the animals treated with the test drug. It shows that the test drug hasn't produced any internal hemorrhage or organ related toxicity.

Sub - Acute toxicity study

Sub-acute toxicity for the given test drug was carried out as per the OECD guideline 407 by repeated dose administration of the test drug in animals and further animals were closely monitored for the emergence of toxicity. Since, there were no significant adverse effects on the haematological, biochemical and histopathological parameters it may be concluded that

the test drug at both the dose level of 200mg/kg and400mg/kg may be considered as relatively safe, as it did not cause either mortality or produce severe toxicological effects on selected body organs, biochemical indices and haematological and histopathological markers of rats during the sub-acute periods of study.

CONCLUSION:

The current toxicity study proves that the recommended dosage of the Siddha formulation *KadalazhinjilKudineer* does not produce any pathological symptoms throughout the dosing period of 28 days. So the safety drug dose of *KadalazhinjilKudineer*is 400mg/kg/body weight.

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