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Full Length Research Paper

ACUTE DERMAL TOXICITY STUDIES OF TROIS™ IN NEWZEALAND WHITE RABBITS

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ABSTRACT

The study was performed to assess the acute dermal toxicity of Trois™ in Newzealand white rabbit. Test substance was applied as such to the shaven skin of group of rabbits at the dose of 2000 mg/Kg body weight. Control group of animals were similarly treated but only with base. Following dosing up to 14 days the rabbits were observed for mortality and clinical sign of toxicity. No visible signs of toxicity after treatment were observed on the animals of both control and treated animals up to 14 days. Various haematological and biochemical parameters were evaluated and found to be in the normal limit, which indicates that no sign of toxicity in NewZealand white rabbits after 14 days treatment in respect to control group, proving safety of Trois™ in topical application.

Key-words: *Arthritis, inflammation, biochemical parameters, medicinal plant.*

INTRODUCTION

Joints pain and swelling are common complaints presented by adult patients. Medicinal oils have been used for the management of joints pain and swelling [1,2]. Medicinal plant may produce several biological activities in human, generally very few are known about their toxicity because safety should be the overriding criterion in the selection of medicinal plants for use in health care systems [3].

The plant based multicomponent formulation has been developed for topical application in patient with joint pain and swelling. Trois™ is a nano-technology based herbal formulation and is combination of various herbal extract. It is effective against different types of arithritis including osteoarthritis, gouty arthritis, rheumatoid arthritis, ankylosing spondylities, psoriatic arthritis, sprain & backage. it gives results in a limited period of time and provide relief from pain. It is used topically by applying at the site of pain. Due to this, it becomes necessary to judge dermal safety of prepared formulation.

In Trois™, plant extracts are incorporated into suitable bases, but there are chances of contamination which may prove toxic to the individuals or may develop any other complications [4]. Hence the present study was taken to investigate the dermal toxicity of Trois™ possessing anti-arthritic and anti-inflammatory activity in rabbits. In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from a short-term exposure by the dermal route. Data from an acute dermal toxicity study may serve as a basis for classification and labelling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route [5,6].

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MATERIALS & METHODS

Animals

Total 10 young male adult NewZealand white rabbit, weighing 1.8-2 Kg were obtained from laboratory's animal house. All animals were divided into two groups (one treatment group and one control group). Each group contains 5 animals. The animals were housed individually in stainless steel cages at controlled room temperature of 27-29 °C and a relative humidity between 30 to 70% with a constant light-dark schedule (12 hours light and 12 hours dark cycle). The animals were maintained according to the recommendation contained in the DHEW Publication No. 86-23 (NIH): "Guide for the Care and Use of Laboratory Animals". Purina rabbit chow and water were available *ad libitum*. The rabbits were acclimated at least 4 days prior to start experiment.

Animal Preparation

Prior to start the experiment, the dorsal and ventral areas of the trunk of the rabbits were shaved, the shaved area were approximately 30% of the total body surface area. The 24 hour period between shaving and application of the material allows recovery of the stratum corneum from any disturbance caused by the shaving.

Test material and treatment levels

The test material is a pink colour nanosuspension possess anti-inflammatory and antiarithritic. Initial testing was performed at a dose level of 2 g/Kg.

Dosing

All the animal were weighed on the day of dosing. Based on the animal's body weight the test material was applied uniformly over the shaved area and covered with two layers of porous gauge dressing. A sleeve of plastic sheeting is fitted over the shaven trunk of the animal and secured in the place with non irritating tape. The test animals are returned to their cages for the 24 h. The test material remaines in contact with the skin for 24 h. The remaining test material was wiped and test material was applied daily once a day for 14th days according to their body weight.

Observation during study

All the test and control animals were observed frequently during the day of dosing and once daily for 14 days following dosing for any toxic and deleterious effects. The primary pharmacotoxic observation were limited to the skin at the application sites and include; erthema, edema and scaling of the epidermis. Physical parameters (body weight and food intake) and local injury were studied throughout the treatment. Mortality if any, in all of the groups, during the course of treatment was also recorded. Autopsy was done if any animal died during the course of treatment. At the end of treatment, blood samples were collected on 15th dayusing sodium citrate (3.8 %) as a anticoagulant. Various haematological and biochemical parameters were studied.

Hematological and Biochemical Parameters

Blood samples were analysed for routine haematological parameters. Blood cell count was done with blood smears. Hemogram was performed on ACT diff-2 Hematolgy Analyzer (Beckman Coulter India, Ltd., Mumbai, India). Biochemical parameters were performed in serum sample. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), alkaline phosphatase (ALP), Total serum protein, and blood sugar levels, uric acid, Bilirubin and creatinine were estimated. All parameters were studied by Merck semi auto analyzer by using Merck analytical kits.

Statistical analysis

The representing data are shown as mean Â± SD. Dunnett's test was performed for the evaluation of data. $P < 0.01$ was considered as significant.

Results and Discussion

There was no significant changes were observed in physical behavioral throughout the experimental period. The group mean body weight of all treated and control animals were not altered significantly. The gross pharmacotoxic observations were found to be normal in all treated and control animals (Table 1 and 2).

In all treated and control animals, there was no any significant alterations were observed in hemoglobin (Hb), red blood cell counts (RBC), white blood cell (WBC) counts and platelet counts as compared to control group (Table 3).

There was no significant changes in SGOT, SGPT and ALP activities were observed in all the treated animals as compared to respective control animals. The levels of total serum proteins and blood sugar levels were not altered in all treated and control animals. There was no significant alteration uric acid, creatinine and bilirubin in all treated and control animals (Table 4).

In conclusion, our results indicate that Trois™ is safe and no significant effects were observed on any of physiological and biochemical parameters.

Table 1: Group mean body weight (Kg) of rabbits

Group No.	Dose mg/Kg	Days		
		0	7	14
I	Control	1.85±0.09	1.87±0.09	1.89±0.09
II	Treated	1.89±0.05	1.91±0.05	1.95±0.05

Table 2: Gross Pharmacotoxic Observations

Hours				Days													
Animal No.	1	2.5	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control																	
1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Treated																	
6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
7	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
8	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
9	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

10	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Note: √ It means normal.

TABLE 3: Hematological parameters in rabbits

Parameters	Control	Treated
Hb (g%)	7.60±0.52	7.65±0.45
Total RBC ($x 10^6$/cmm)	6.85±0.53	7.25±0.22
Platelets ($x 10^5$cmm)	3.04±0.18	3.12±0.12
Total WBC ($x 10^3$cmm)	6.91±0.45	7.11±0.54

TABLE 4: Biochemical parameters in rabbits

Parameters	Control	Treated
Total serum Protein (g%)	1.46±0.03	1.54±0.06
Urea (mg%)	5.53±0.02	5.52±0.01
SGPT (IU/L)	63.71±0.73	63.07±2.93
SGOT (IU/L)	96.07±0.49	95.01±0.84
ALP (IU/L)	14.04±1.00	14.02±0.67
Blood sugar (mg%)	102±5.43	102±6.44
Uric acid (mg/dL)	1.83±0.01	1.86±0.02
Creatinine (mg/dL)	0.22±0.01	0.25±0.01
Bilirubin (mg/dL)	0.67±0.15	0.64±0.13

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