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Research Article

ACUTE TOXICITY STUDY OF ARTILON SOFT GEL CAPSULE IN SWISS ALBINO MICE

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ABSTRACT

Artilon Soft Gel capsule is a herbal formulation based on Ayurvedic principles used as anti-inflammatory. The purpose of the study was to test the acute oral toxicity of the drug in Swiss Albino mice. The studies were carried out based on OECD guidelines 423 and fixed dosage studies was adopted where the limit dose is 2000 mg/kg body weight of test animal. The animals were orally administered a single dose of 2000 and 5000 mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the drug for 14 days. The highest dose administered (5000 mg/kg body weight) did not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of Artilon Soft Gel capsule.

Keywords: Artilon Soft Gel capsule, Acute toxicity, LD50, Mice, Ayurveda, Herbal.

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization[1]. Thousands of years ago an extensive use of herbs as medicines have been reported and were initially taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other herbal formulations[2]. The processes and methods developed with the passing of time and so does the evaluations of the formulations. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Whenever we administer a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. So the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs if administered injudiciously. A typical example for a toxic herbal product used also in Ayurvedic formulation are the leaves of *Atropa Belladonna*[3] and *Digitalis purpurea*[4], which shows severe systemic toxicity if taken orally.

Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to the use as drug or chemical in clinical use[5]. As per the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey, etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 & 425 does not allows the use of drug clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animal's toxicological studies may be three types such acute, sub-acute and chronic toxicological studies.

In **acute toxicity** studies, single dose of drug given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products.

In **sub-acute toxicity** studies, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues.

In **chronic toxicity studies**, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic potential of drug[6].

Plants or drugs must be ensured safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to

conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used[7]. The present study has been undertaken to estimate the toxic effects of Artilon Soft gel capsule, a proprietary formulation developed by Nagarjuna Herbal Concentrates Ltd., Kerala, in Swiss Albino mice (female) at the dosage of 2000, 5000mg/kg body weight of an animal for a period of 14 days using OECD 423.

MATERIALS AND METHODS

1. Preparation of Medicine

All the ingredients used for this medicine were collected from raw material store of Nagarjuna Herbal Concentrats Ltd, Thodupuzha, Kerala, India. The herbs were identified and authenticated at the Herbarium of Pharmacognosy department, Nagarjuna Herbal Concentrats Ltd, Thodupuzha, Kerala, India. The process is as per the preparation of Aavarthy (Repeating the process), a pottenciation process mentioned in the Ayurvedic classical texts.

All the raw materials were cleaned, washed and dried. The 17 raw materials used for preparing the *Kashayam* (decoction) were divided into 14. The 32 raw materials for *Kalkam* (Paste) are powdered and also divided into 14 parts. From these, 3 parts were taken together for preparing the decoction for three *Avarthees* at a time. This was then added with 16 times of water and reduced to one fourth through boiling. On completion it was filtered and divided into three parts. One part of this kashayam along with *Nallenna* (Sesame oil) and one part of dried powder were boiled up to the proper consistency (*Chali pakam*). When the consistency is reached, milk is added up to thrice the quantity of oil and boiled again till it reaches the next consistency (*Chikkana pakam*). Then this was filtered and allowed to settle the sediments and filtered. This process was repeated up to 41 times with the remaining materials in the same oil. The final product was filtered till all the residues were separated and used for the study.

2. Experimental Animals

Animals were selected as per the OECD guidelines. Healthy young and non pregnant Swiss Albino female mice weighing from 24g were selected, because literature survey of LD50. Test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive[8]. The animals bred in Nagarjuna Herbal Concentrates Ltd., Thodupuzha, Kerala, India, were used. All animal experiments were approved by the Institutional Animal Ethics Committee, Nagarjuna Herbal Concentrates Ltd. and were maintained in accordance with the guidelines of the CPCSEA. The animals were housed in polypropylene cages in a room with a 12 hour day-night cycle and were maintained on a standard pelleted feed and free excess of water was given *ad libitum*. To acclimatize with the laboratory conditions, randomly selected animals marked to permit individual identification were kept in clean polypropylene cages for 5 days prior to start of experiment.

3. Methodology

Paragraph 22 of OECD Guideline 423 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test is performed.

Paragraph 23 of OECD Guidelines suggests a limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

Test substance administration volume

The administration volume was below 1 ml/animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Test Procedure

Prior to the dosing, the animals were fasted overnight for 24 hours. Following the period of fasting, the fasted body weight of each animal was determined as stated in paragraph 26 of OECD Guidelines 423 and the dose was calculated according to the body weight as per the Annex 2d of OECD Guidelines 423 and as stated in Paragraph 23 of OECD Guidelines 423[8].

As per OECD Guidelines the starting dose of 2000 mg/kg body weight of an animal was used and prepared at 200 mg/ml of Tween 20 as diluents. The medicine was given to three animals as first step. After the medicine was orally administered, animals were observed keenly for about 48 hours, no mortality was observed. So later given same dose to next three and done as above. No mortality was observed and given next dose of 5000mg/kg in two steps for 6 animals as above and observed. Normal control group was discarded as per the suggestions of IAEC. The animals were observed for 2 weeks. Daily water and food intake and weekly body weight changes were noted. On 14th day the animals were sacrificed and done detailed necropsy.

4. Observations

As per the Paragraph 24 and 25 of OECD Guidelines 423, Wellness parameters of animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded. Observations included changes in skin and fur, eyes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Further Individual body weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated as stated in the Paragraph 26 of OECD Guidelines 423.

RESULTS AND DISCUSSION

The present study conducted as per the OECD guidelines 423 revealed that the Artilon Soft Gel capsule did not produce any mortality throughout the study period of 15 days even at higher dose of 5000 and 2000 mg/kg body weight. No significant changes were observed in body weight and wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation and sleep of the treated animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animals (table I). Although, the body weight of all the mice were increased after the oral administration of medicine (table II). The increase in body weight of test animals indicates that the administration of the Artilon Soft Gel capsule does not affect the growth of the animals. Water and food intake of the animals were also in normal condition (table III). The viscera of the animals did not show any macroscopic changes at necropsy on study day 14. The animals internal organs weight were shown in table IV.

Ayurveda is an ancient science and herbal medicines have received a great attention as alternatives to synthetic pharmaceutical products in recent times, leading to the increase in their demand[9]. Medicinal plants presence of phytochemicals like flavonoids, alkaloids, sterols, proteins, anthroquinones and phenolcs. These metabolites are generally used in various pharmaceutical and cosmetic preparations which are an indication that these metabolites may be non-toxic. The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common in rural communities. Experimental screening method is, therefore, important to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies[10].

The median acute toxicity value (LD50) of Artilon Soft gel Capsule was determined more than 5000 mg/kg body weight. However, since the animals did not convulse, it could be postulated that mortality did not result from some action of the extract on the nervous system[11]. The increase in weight was remarkable in the treated animals. This was clearly shown by the mean percentage increase in the weights of the treated animals. The observed increase in the weights might be attributed to the appetite stimulation effect of the Artilon Soft gel Capsule on the animals.

CONCLUSION

The non-toxic nature of the Artilon Soft Gel Capsule is evident from the acute oral toxicity conducted as per OECD guidelines. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid drug. Hence Artilon Soft Gel Capsule could be safe up to the dose of 5000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxic symptoms.

LD50 Value: As per observations and calculations from Acute Oral Toxicity (OECD Guidelines 423), the LD50 value of Artilon Soft gel Capsule was found to be **more than 5000mg/kg** body weight.

Table I - Effect of Artilon Soft Gel capsule on Swiss Albino Mice at 2000 and 50000mg/kg body weight observations

Observations	30min		4hrs		24hrs		48hrs		1 week		2 week	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Skin and Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table II - Effect of Artilon Soft gel capsule on body weight of Swiss albino mice at 2000 mg/kg body weight and 5000 mg/kg body weight

Groups	Dose (mg/kg)	Initial (gm)	1st week (gm)	2nd week (gm)
Group I	2000	24.0±0	25.0±0.8	26.6±1.0
Group II	5000	24.0±0	25.0±1.0	27.0±1.0

Values are given as mean ± S.D.E for six rats in each group.

Table III: Effect of Artilon Soft gel capsule on food and water intake of Swiss albino mice at 2000 mg/kg body weight and 5000 mg/kg body weight

Groups	Dose (mg/kg)	1st week (ml)		2nd week (gm)	
		Food	water	Food	water
Group I	2000	4.4±0.7	4.2±0.53	4.3±0.5	6.0±1.2
Group II	5000	4.7±0.2	4.5±0.2	4.4±0.3	5.3±1.2

Values are given as mean ± S.D.E for six rats in each group.

Table IV: Effect of Artilon Soft gel capsule on internal organs of Swiss albino mice at 2000 mg/kg body weight and 5000 mg/kg body weight

Groups	Dose (mg/kg)	Liver (g)	Kidney (g)	Heart (g)	Lungs (g)
Group I	2000	0.83±0.03	0.168±0.01	0.101±0.005	0.146±0.02
Group II	5000	0.81±0.03	0.167±0.0	0.102±0.006	0.148±0.01

Values are given as mean ± S.D.E for six rats in each group.

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