Lab No.:_____

P.O. No.: 2001051**0**

PRODUCTS EFFICACY

LABORATORY REPORT

Test Unit:

Dept. of Pharmacology
School of Pharmaceutical Science
Jilin University
8 Xinmin St.
Changchun, JiLin, China

Sponsor:

Hamida Pharma, Inc.
15041 Bake Pkwy, #E
Irvine, CA92618
U.S.A.

TEST TITLE:

ANTI-RHEUMATIC ARTHRITIS EFFICACY STUDY OF ARTHRITOL (ANTI-ADJUVANT-INDUCED ARTHRITIS TEST)

TEST ARTICLE: ARTHRITOL

Source: Hamida Pharma, Inc., USA

Identification No.: AR-1003

STUDY & SUPERVISORY PERSONNEL:

Shaochun Qu, Xiaofeng Yu, Dayuan Sui

STUDY DIRECTOR:

Xiaofeng Yu, B.M. _____ Date: May, 2002

Study director

APPROVED BY:

Prof. Dr. Dayuan Sui _____ Date: May, 2002

Director of Dept. of Pharmacology

ABSTRACT:

Freund's Adjuvant-Induced Arthritis on rats is a delayed type allergythat is similar to the rheumatic arthritis on human being and often used for Anti-Rheumatic Arthritis study. This study of Arthritol was carried out according to principles of traditional Chinese medicine study. No matter in prevention groups or in treatment groups of Arthritol, the study results showed that Arthritol could not only inhibit inflammatory reaction on both early stage and later stage, but also inhibit delayed type allergy. The efficacy of Arthritol at both 2g/kg and 4g/kg can match that of Aspirin.

OBJECT: To evaluate the potential anti-rheumatic properties of Arthritol against the inflammatory and delayed type allergy induced by Freun's adjuvant injected into the sub-plantar right hind paw of a rat. This test is one of the standard methods for evaluating anti-rheumatic activity.

TEST ARTICLE:

Arthritol powder supplied by Hamida Pharma, Inc. of the United States.

Identification No.: AR-1003

GENERAL SYSTEM PARAMETERS:

Animals:

Species: Wistar rats

Source: Experimental Animal Center of Jilin University

Certificate: 10-5112

Sex: Male and Female

Age: No particular age was prescribed for this study

Body Weight Range: 180-220 gram

Acclimation Period: 2 days

Number of Animals: 50

Identification Method: fur coloring

Animals Management:

Husbandry: Conditions conformed to Standard Operating Procedures, which are based on the "Guide for the Care and Use of Laboratory Animals".

Food: NIH-07 Rodent diet was provided daily.

Water: Freely available, municipal water was delivered through an automatic watering system.

Contaminants: Reasonably expected contaminants in food or water supplies did not have the potential to influence the outcome of this test.

Housing: Animals were housed in groups of 10 per extract in stainless steel cages identified by a card indicating the animal numbers, test code, sex, animal code and date dosed.

Environmental: The room temperature was monitored daily. The temperature range for the room was within a range of 20-26℃. The room humidity was monitored daily. The humidity range for the room was 40-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark.)

Facility: Pharmacology Lab of Basic School of Medical Science, Jilin University is registered with the Ministry of Public Health of PRC and conforms to GLP standards.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused animals were selected.

Test System Justification:

This test is a standard method for evaluation of anti-adjuvant-induced arthritis activity and there is a good correlation to anti-rheumatic arthritis activity in man. Compounds working in this test may be considered to have activity comparable to non-steroidal anti-inflammatory drugs. (NSDAIDs).

METHOD:

The 70 rats were randomly and equally distributed into 7 groups (10 rats/group), namely, 1) Vehicle Control Group, 2) 2g/kg of Arthritol Prevention Group, 3) 4g/kgof Arthritol Prevention Group, 4) 2g/kg of Arthritol Treatment Group, 5) 4g/kg of Arthritol Treatment Group, 6) 200mg/kg of Aspirin Prevention Group, and 7) 200mg/kg of Aspirin Treatment Group. After ether anesthesia, the rats were injected 0.05ml of Freun’s adjuvant (including 0.5mg of mortuary mecobacterium tuberculosis) into the right-hind food-plate. At the same day, the rats in Prevention Groups were administered Arthritol or Aspirin orally using appropriate size needles

and syringes, 1 time a day, for 21 consecutive days. From 8th day, the rats in Treatment Group were administered Arthritol or Aspirin orally 1 time a day, until 21st day. The rats in Vehicle Control Group were administered physiological saline orally, 1 time a day, from 1st day till 21st day. After administering, all rats were observed for another 3 days. At 24th day, all rats were killed and made dissection, weighing thymus, spleens and adrenal. The volumes of left and right hind paws were measured in 3 hours of injection of Freund's adjuvant and every a few days. The difference of the volumes of Rats' paws between before and after administering is the degree of swelling. Otherwise, the red patches on the rats' ears and tubers on the tails should be observed at anytime. The rats were weighed every 3 days.

RESULTS:

Preventive Administering: 2g/kg and 4g/kg of Arthritol does not only inhibit early inflammatory reaction on the local area of injection and the re-swelling in 12 days, but also inhibit the paw swelling induced by delayed type allergy on the other side of rat's paws. The strength is roughly similar to Aspirin.

Therapeutic Administering: 2g/kg and 4g/kg of Arthritol notably reduced the swelling and re-swelling of the paws on which made injection, and 4g/kg of Arthritol could also observably inhibit the swelling induced by delayed type allergy. (See Table-2).

Others: Comparing with Vehicle Control Groups, the red patches on the rats' ears and tubers on the tails in both Arthritol groups and Aspirin Groups are reduced obviously. During the whole period of study, food intakes of rats were not obviously reduced. Arthritol did not show to affect the weights of rats' bodies, and also thymuses, spleens and adrenals.

CONCLUSION:

A statistically significant decrease in the paw volume in prevented and treated rats by Arthritol compared to that of vehicle control is considered that Arthritol have anti-inflammatory and anti-rheumatic arthritis activity comparable to non-steroidal anti-inflammatory drugs (NSDAIDs), such as Aspirin.

RECORD STORAGE:

All original data pertaining to this study and a copy of the final report are to be retained in Dept. of Pharmacology, School of Pharmaceutical Science, Jilin University of China.

Table 2. Effects of Arthritol on Rats'Adjuvant-induced Arthritis（n=10，x±s）

Groups	Degree of Swelling at different day after injection of Freund's Adjuvant（ml）								
	3h	2d	5d	8d	12d	15d	18d	21d	23d
INJECTED FOOT (RIGHT)									
Vehicle Group	1.21±0.38	1.10±0.24	1.23±0.37	1.25±0.35	1.43±0.24	1.85±0.38	2.07±0.51	2.39±0.68	2.14±0.65
Asprin Prevention Group									
0.2g/kg	1.04±0.32	0.84±0.28*	0.76±0.28**	0.94±0.25*	0.83±0.26***	1.10±0.31***	1.32±0.39**	1.76±0.54*	1.74±0.45
ARTHRITOL Prevention Group									
2g/kg	1.10±0.41	0.98±0.32	0.87±0.31*	0.96±0.24*	0.98±0.29**	1.40±0.47*	1.58±0.44*	1.96±0.54	2.04±0.35
4g/kg	1.07±0.28	0.90±0.33	0.71±0.38**	0.85±0.31*	0.92±0.31***	1.07±0.48***	1.28±0.41**	1.72±0.46*	1.65±0.43
Asprin Therapeutic Group									
0.2g/kg	1.30±0.33	1.18±0.31	1.26±0.51	1.24±0.45	0.98±0.37**	1.13±0.36***	1.34±0.47**	1.79±0.41*	1.85±0.31
ARTHRITOL Therapeutic Group									
2g/kg	1.20±0.34	1.12±0.34	1.19±0.48	1.27±0.57	1.09±0.34*	1.40±0.41*	1.63±0.36*	1.78±0.48*	1.82±0.55
4g/kg	1.26±0.37	1.14±0.42	1.23±0.39	1.28±0.47	1.06±0.39*	1.26±0.44**	1.42±0.34**	1.56±0.47**	1.71±0.45
UNJECTED FOOT (LEFT)									
Vehicle Group					0.23±0.12	0.21±0.13	0.36±0.15	0.65±0.18	0.61±0.17
Asprin Prevention Group									
0.2g/kg					0.09±0.16*	0.08±0.11*	0.12±0.21**	0.36±0.26*	0.37±0.25*
Asprin Prevention Group									
2g/kg					0.14±0.29	0.09±0.17	0.18±0.16*	0.39±0.14*	0.34±0.31
4g/kg					0.10±0.13*	0.04±0.18*	0.10±0.19**	0.41±0.20*	0.29±0.30**
Asprin Therapeutic Group									
0.2g/kg					0.15±0.21	0.10±0.08*	0.15±0.17**	0.52±0.21	0.45±0.24
ARTHRITOL Therapeutic Group									
2g/kg					0.19±0.14	0.13±0.24	0.23±0.22	0.48±0.38	0.41±0.35
4g/kg					0.16±0.17	0.09±0.10*	0.16±0.14**	0.32±0.27**	0.37±0.32*

Comparing with Vehicle Control Group, *p<0.05, **p<0.01, *** p<0.001