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TOXICOLOGICAL REPORT

COPY

ON

ACUTE ORAL AND ACUTE DERMAL TOXICITY OF STREPTOMYCES GANOSA1 IN RATS.

Prepared for

Malaysian Palm Oil Board
6, Persiaran Institusi
43600 Kajang
Selangor
MALAYSIA.

Prepared by

Prof. (Dr.) Mohd. Zaini bin Asmawi and Associates
School of Pharmaceutical Sciences
Universiti Sains Malaysia
Penang, MALAYSIA.
12th December 2012.

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UNIVERSITI SAINS MALAYSIA
PUSAT PENGAJIAN SAINS FARMASI
SCHOOL OF PHARMACEUTICAL SCIENCES

MEMORANDUM

Ref : PPSF/TR/1164

TOXICOLOGICAL REPORT ON ACUTE ORAL AND ACUTE DERMAL TOXICITY OF STREPTOMYCES GANOSA1 IN RATS.

Sponsor :

Malaysian Palm Oil Board
6, Persiaran Institusi
43600 Kajang
Selangor
Malaysia.

Test Substance : Streptomyces GanoSA1, small brownish granules with silver flakes, is supplied by the client.

Method used : Acute Oral Toxicity (Up-and-Down Procedure):
As stipulated by:-
OECD Guideline for Testing of Chemicals 425, 2001

Acute Dermal Toxicity:
As stipulated by:-
OECD Guideline for Testing of Chemicals 402, 1987

Results :

The estimated LD_{50} of Streptomyces GanoSA1 p.o. were 2000 mg/kg in female rats. The LD_{50} for dermal administration were >2000 mg/kg in male rats and >2000 mg/kg in female rats.

Summarised results are presented in the tables below (attached). Detailed data for each rat used in the studies are documented in the appendices.

Finally, this is to certify that the test had been carried out in compliance with Good Laboratory Practice Standards.

Study Director,

Professor Mohd. Zaini bin Asmawi

11800 USM , Pulau Pinang, Malaysia
Tel : (6)04-653 2211, 653 2212, 653 2161, 653 2229; Fax : (6)04-657 0017; Website : www.usm.my; www.pha.usm.my

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A. Animals:

Sprague-Dawley rats obtained from Animal Research And Service Centre (ARASC), Universiti Sains Malaysia, 11800 Penang, Malaysia, were quarantined for a week before use. The animals both for oral and dermal toxicity studies were housed individually.

The animals were kept at room temperature with 12-hr light/dark cycle. Each animal had free access to water and rat feed. The rat feed was obtained from Gold Coin Malaysia Sdn. Bhd. Butterworth, Penang, Malaysia.

B. Test Substance:

Streptomyces GanoSA1, small brownish granules with silver flakes supplied by the client was grinded & sieved before suspended in tragacanth mucilage (B.P.C) 25%. Dosing was 10ml/kg for oral administration and 5ml/kg for dermal.

C. Procedure:

C.1 Acute oral toxicity study.

[No. of Animal Ethic Approval: USM/Animal Ethics Approval/2010/(56)(188)].

The acute oral toxicity study was conducted using the OECD Guideline For Testing Of Chemicals 425, 2008.

Female rats (nulliparous and non-pregnant) are used for oral toxicity study. First, a female rat is fasted overnight and administered with a single oral dose of Streptomyces GanoSA1. The dose given is a step below the best preliminary estimate of the LD50. The animal is observed closely for any symptom of toxic effects. The onset and duration of the toxic symptom observed are recorded. If the first animal died, a second animal was administered with even a lower dose of Streptomyces GanoSA1. If the first animal survived for more than 48 hours, the second female rat is administered with a higher dose and observed as before. Similarly, if the second animal died, the third female rat is administered with a lower dose of Streptomyces GanoSA1. If the second animal survived for more than 48 hours the third female rat is administered with a higher dose of Streptomyces GanoSA1. Treatment of an animal at the next dose is delayed until one is confident that the previously dosed animal will survive. The time interval for dosing may be adjusted. The sequence of giving higher and lower doses is continued until data on five rats are obtained. The animal that survived the dosing is continued to be observed at least once a day for 14 days before being sacrificed for necropsies.

The LD_{50} value for oral administration of the test substance was determined using the software AOT425StatPgm Program. (1).

C.2 Acute dermal toxicity study.

[No. of Animal Ethic Approval: USM/Animal Ethics Approval/2010/(56)(189)].

The acute dermal toxicity study was conducted using the OECD Guideline For Testing Of Chemicals 402, 1987.

The dorsal area of the trunk of two groups of 5 male rats and two groups of 5 female rats (nulliparous and non-pregnant) were shaved approximately 24 hours before the study. The test substance was applied at the dose of 2.0 g/kg uniformly on the shaved part, covering about 10% of the body surface area. This area was then covered with semi permeable film such as opsite or tegarderm and held in contact to the skin with non-irritant tape. 24 hours after the control or test substance application, the tape and semi permeable film were removed and the exposed skin of the treated area was washed with water (to remove the residual test substance).

The toxic sign and symptom of the dermally treated animals were closely monitored on the first day and at least once a day for the remaining 14 days.

In the limit test if all the 5 animals of the same sex survived, the LD50 is considered higher than 2000 mg/kg for that particular sex. If compound-related mortality is produced, a full study is warranted. In the full study, at least five males and five females are used for each dose level. At least three dose levels are used and spaced appropriately to produce test group with a range of toxic effects and mortality rates. The data obtained should be sufficient to produce a dose-response curve and where possible, permit an acceptable determination of the LD50. The LD50 value for dermal administration of the test substance would be determined using probit analysis (Finney, 1952) (2).

D. Results:

Table 1 shows that none of the female rats died at the end of the second week after oral (175, 550 and 2000 mg/kg) administration of Streptomyces GanoSA1. The experiment was repeated 3 times with 2000 mg/kg p.o Streptomyces GanoSA1 and none of the animal died.

Oral administration of 175mg/kg, 550 mg/kg and 2000mg/kg Streptomyces GanoSA1 did not cause any toxic symptoms (Table I). The female rats gain weight every week. The maximum oral dose for Streptomyces GanoSA1 was 2000mg/kg as the sample is very light and it was very difficult and almost impossible to prepare suspension for oral administration higher than 2000 mg/kg. The suspension became very thick and difficult to flow through the oral needle.

Calculation of LD50 using software program "AOT425 StatPgm" showed that the oral LD50 of Streptomyces GanoSA1 is more than 2000 mg/kg (Table II).

After 14 days of study and monitoring, each of the animals was subsequently sacrificed for nepcrosy. There were no internal organs changes observed in any of the Streptomyces GanoSA1 orally treated rats.

None of the male and female rats died at the end of the second week after dermal (2000mg/kg) administration of Streptomyces GanoSA1 (Table III and IV) . This suggests that the LD50 for dermal administration for Streptomyces GanoSA1 were more than 2000 mg/kg in the male rats and more than 2000 mg/kg in the female rats (Table V). There was no toxic sign and symptoms observed during the two weeks observation period in both the male and female rats (Table III and IV). Necropsy studies done two weeks after treatment also did not show any physical changes in the rats' organs. All the dermally treated rats gain weight every week similar to the control group (Table VI).

E. Discussions:

Cage side observations and necropsy studies did not detect any external nor major physical organ tissues abnormality caused by Streptomyces GanoSA1

The results show that the LD50 of orally administered Streptomyces GanoSA1 in female rats is higher than 2000 mg/kg.

There was no toxic sign and symptoms observed when the male and female rats were dermally treated with 2000 mg/kg Streptomyces GanoSA1. This suggests that the LD50 of dermally administered Streptomyces GanoSA1 is more than 2,000 mg/kg in both the male and female rats.

F. Conclusion:

The oral LD50 of Streptomyces GanoSA1 on female rats is >2000 mg/kg. The LD50 for dermal administration were >2000 mg/kg for both the male and female rats.

G. References:

(1) Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT 425 StatPgm). Version:1.0, 2001.

(2) Finney D.G., Probit Analysis, 2nd Edition Cambridge University Press, Cambridge, (1952).

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TABLE I : The effect of oral administration of different doses of Streptomyces GanoSA1 on female rats body weight, toxic symptoms and internal organs.

Rat no.	DOSE (mg/kg)	Weight of rat (g) week 0	week 1	week 2	at death	Onset of Death (h)	Toxic Symptoms	Internal organs
1	175	216	240	247	---	---	Normal.	All organs normal.
2	550	206	220	231	---	---	Normal.	All organs normal.
3	2000	210	216	219	---	---	Normal.	All organs normal.
4	2000	225	228	231	---	---	Normal.	All organs normal.
5	2000	220	221	236	---	---	Normal.	All organs normal.

TABLE II: The effect of oral administration of Streptomyces GanoSA1 on female rats mortality.

Test seq.	Animal ID.	DOSE (mg/kg)	Mortality Short-term result. (in 48 hours)*	Long-term result. (in 14 days)*	Estimated LD50 (mg/kg)	95% Confidence Interval (mg/kg)
1	1	175	O	O	> 2000	---
2	2	550	O	O		
3	3	2000	O	O		
4	4	2000	O	O		
5	5	2000	O	O		

*Survive=O *Death=X

Summary of Long-Term Results:

Dose	Survive	Death	Total
175	1	0	1
550	1	0	1
2000	3	0	3
All Doses	5	0	5

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TABLE III: The effect of dermal administration of 2000 mg/kg Streptomyces GanoSA1 on male rats body weight changes, toxic symptoms and internal organs.

DOSE (mg/kg)	Weight of rat (g) week 0	week 1	week 2	at death	Onset of Death (h)	Toxic symptoms	Internal Organs
2000	237	262	282	---	---	Normal.	All organs normal.
	232	249	255	---	---	Normal.	All organs normal.
	251	264	275	---	---	Normal.	All organs normal.
	282	316	336	---	---	Normal.	All organs normal.
	283	294	305	---	---	Normal.	All organs normal.
control	288	305	311	---	---	Normal.	All organs normal.
	273	288	308	---	---	Normal.	All organs normal.
	251	269	318	---	---	Normal.	All organs normal.
	238	244	271	---	---	Normal.	All organs normal.
	249	261	281	---	---	Normal.	All organs normal.

TABLE IV: The effect of dermal administration of 2000 mg/kg Streptomyces GanoSA1 on female rats body weight changes, toxic symptoms and internal organs.

DOSE (mg/kg)	Weight of rat (g) week 0	week 1	week 2	at death	Onset of Death (h)	Toxic symptoms	Internal Organs
2000	226	245	254	---	---	Normal.	All organs normal.
	191	199	201	---	---	Normal.	All organs normal.
	214	227	236	---	---	Normal.	All organs normal.
	228	247	250	---	---	Normal.	All organs normal.
	193	200	230	---	---	Normal.	All organs normal.
control	213	219	229	---	---	Normal.	All organs normal.
	199	209	231	---	---	Normal.	All organs normal.
	209	218	227	---	---	Normal.	All organs normal.
	237	244	255	---	---	Normal.	All organs normal.
	220	228	235	---	---	Normal.	All organs normal.

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TABLE V : The effect of dermal administration of 2000 mg/kg Streptomyces GanoSA1 on male and female rats mortality.

ROUTE	SEX	DOSE (mg/kg)	Mortality: Death/ Numbers	Onset of death (Hrs): Mean	Onset of death (Hrs): Std Err	LD50 (mg/kg)	95% Confidence Interval (mg/kg)
DERMAL	MALE	2000	0/5	---	---	>2000	---
		control	0/5	---	---		
	FEMALE	2000	0/5	---	---	>2000	---
		control	0/5	---	---		

TABLE VI :The effect of dermal administration of 2000 mg/kg Streptomyces GanoSA1 on mean body weight of male and female rats.

ROUTE	SEX	DOSE (mg/kg)	Initial: Wt (g)	Initial: Std Err	Week 1: Wt (g)	Week 1: Std Err	Week 2: Wt (g)	Week 2: Std Err	at death: Wt (g)	at death: Std Err
DERMAL	MALE	2000	257	10.9	277.0	12.2	290.6	13.9	---	---
		control	259.8	9.1	273.4	10.6	297.8	9.2	---	---
	FEMALE	2000	210.4	7.9	223.6	10.4	234.2	9.4	---	---
		control	215.6	6.3	223.6	5.9	235.4	5.1	---	---