Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2317

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (3):** 73-79 (2016)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Acute Oral Rat Toxicity Study of Multi-herbal formulation (ECD0058)

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Received: 20.06.2016 | Revised: 28.06.2016 | Accepted: 30.06.2016

ABSTRACT

Background and objective: The current study was designed to study acute oral toxicity study of multi-herbal formulation ECD0058 (M/S Enki Lifescience Limited, Mumbai, India) according to OECD guidelines. For new substances it is the recommended stepwise testing approach for developing scientifically sound data on the safety of the substance.

Conclusions: 12 female Wistar rats were selected for acute oral toxicity study of ECD0058. The animals were fasted for minimum 16 hours prior to dosing and for 4 hours post dosing, feed was withheld but drinking water provided ad libitum. 3 rats of the first group G1 were dosed with starting dose of 300 mg/kg body weight and the animals did not show any mortality. So another 3 animals of the same group G1 were dosed with 300 mg/kg body weight and no mortality was observed. Next, 3 animals of group G2 were dosed with 2000 mg/kg body weight and no mortality was observed. So, another 3 animals of the same group G2 were dosed with 2000 mg/kg body weight and no mortality was observed. Hence, further dosing was stopped.

At 300 and 2000 mg/kg body weight, all the animals were observed normal throughout the experimental period.

Key words: *Enki Lifescience Limited, Toxicity, Rat*

INTRODUCTION

Toxicology is the scientific study of adverse effects that occur in living organisms due to chemicals. It involves observing and reporting symptoms, mechanisms, detection and treatments of toxic substances, in particular relation to the poisoning of humans. It includes environmental agents and chemical compounds found in nature, as well as pharmaceutical compounds that are synthesized for medical use by humans. These substances may produce toxic effects in living organisms including disturbance in growth patterns, discomfort, disease and death[7].

The most important stage in ensuring the safety of drugs is to perform toxicity tests in correct animal models[5]. The acute oral toxicity test in rodents aims at establishing the therapeutic index, i.e. defined as the ratio LD_{50}. In general, the narrower this margin, the more likely it is that the drug will produce unwanted effects, the greater the index the safer the compound. However, the term acute oral toxicity is most often used in connection to lethality and lethal dose determinations[2].

Cite this article: Thomas, R. and Kale, P.G., Acute Oral Rat Toxicity Study of Multi-herbal formulation (ECD0058), *Int. J. Pure App. Biosci.* **4(3):** 73-79 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2317

Plants possess various secondary metabolites with profound antimicrobial properties hence they have been used extensively since years. Many herbal based products are being formulated and are brought into the market to prevent infections in hospitals, food service industry, R & D laboratories, etc. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are searching for phytochemicals and "leads" which could be developed into antimicrobials. Many plant extracts have been screened for their antimicrobial potential as it is very likely that these phytochemicals will find their way into collection of antimicrobials which may be used as disinfectants and antiseptics agents[3]. The evaluation of the toxic action of these plant extracts or herbal formulations is important in order to consider them safe before desired usage[4].

The most commonly used herbs with disinfectant properties are *Azdirachta indica, Eucalyptus robusta, Aloe barbadensis, Aloe vera, Withania somniferum, Andrographis paniculata, Aegle marmelos, Berberis vulgaris, Cinnamomum verum, Piper nigrum, Rhamnus purshiana, Capsicum annuum, Syzygium aromaticum, Eucalyptus globulus, Gaultheria procumbens, Cassia angustifolia, Cassia fistula, Mentha piperita* etc. These plants with proven disinfectant activity are also referred to as plant antiseptics[1]. Some of the plants possessing disinfectant properties are enlisted in Table No: 1.

Table no: 1. Active Ingredients of some plants possessing disinfectant properties

NAME OF THE PLANTS	ACTIVE PLANT INGREDIENTS
Glycyrrhiza glabra	Glabrol, Phenolic alcohol.
Garcinia mangostana	Xanthone derivatives, Mangostins.
Galium odoratum	Coumarins.
Hibiscus sabdariffa	Flavonoids, Polyphenols.
Hydrastis Canadensis	Alkaloids, Berberine, Hydrastine.
Hypericum perforatum	Anthraquinones, Hyperecin.
Lawsonia inermis	Phenols, Gallic acid.
Matricaria chamomilla	Phenolic acid, Anthemic acid.
Matricaria recutita	Terpenoids, Flavonoids, Coumarins.
Mentha piperita	Terpenoids, Menthol.
Nelumbo nucifera	Quercetin, Myricetin, Kaempferol, Luteolin.
Ocimum basilicum	Terpenoids, Essential oils.
Olea europaea	Aldehyde Hexanal.
Panax notoginseng	Saponins.
Piper nigrum	Piperine Alkaloid.
Piper betel	Catechols, Eugnol, Essential oils.
Punica granatum	Organic acids, Phenolic compounds.
Quercus rubra	Tannins, Polyphenols.
Rhamnus purshiana	Tannins Polyphenols, Anthraquinones.
Azdirachta indica	Triterpenes, Azadirachtin.
Anethum gravveolens	Essentialoils, Phellandrene, limonene, anithofuran.
Anthemis Nobilis	Terpenoids, Flavonoids, Coumarins.
Andrographis paniculata	Andrographolides, Arabinigalactan proteins.
Aegle marmelos	Essential oil, Terpenoids.
Arctium lappa	Polyacetylene, Tannins, Terpenoids.
Allium sativum	Allicin, Ajoene, Sulfoxide sulfated Terpenoids.
Allium cepa	Allicin.
Artemisia dracunculus	Caffeic acids, Tannins, Terpenoids.
Berberis vulgaris	Berberine Alkaloid.
Cassia fistula	Anthraquinones, Fistulic acid.
Cinnamomum verum	Essential oils, Terpenoids, tannins.
Capsicum annuum	Capsaicin, Terpenoids.
Cassia angustifolia	Rhein, Anthraquinones.
Curcuma zedoaria	Curcuminoids, Demethoxycurcumin, Terpenes.
Carum carvi	Coumarins.
Centella asiatica	Terpenoids, Asiaticoside.
Camellia sinensis	Flavonoids, Catechin.
Citrus paradise	Terpenoids.
Eucalyptus globulus	Tannins, Polyphenols, Terpenoids.
Ficus religiosa	Tannins, Saponins, Flavonoids, Terpenoids.
Gaultheria procumbens	Tannins, Polyphenols.

The objective of the current experiment was to study the Acute oral toxicity of ECD0058 (M/S Enki Lifescience Limited, Mumbai, India) in wistar rats before marketing the product. ECD0058 is a multi-herbal formulation having ingredients viz. *Cymbopogon Citratus, Azadirachta indica, Citronolla Java, Soyabean Oil, Nepeta cataria, Clove Oil, Peppermint Oil* etc. recommended for multi-purpose use like Hygienic general sanitization product, as spray to mask foul smell and to repel flies & insects and to clean hard surfaces as a cleaner concentrate.

MATERIALS AND METHODS

Multi-herbal formulation ECD0058 was supplied by M/S Enki Lifescience Limited, Mumbai, India) to to assess the toxicity potential. Acute oral toxicity study of "ECD0058" was performed as per OECD Guideline 423[5] at SA-FORD, Navi Mumbai, Maharashtra, India

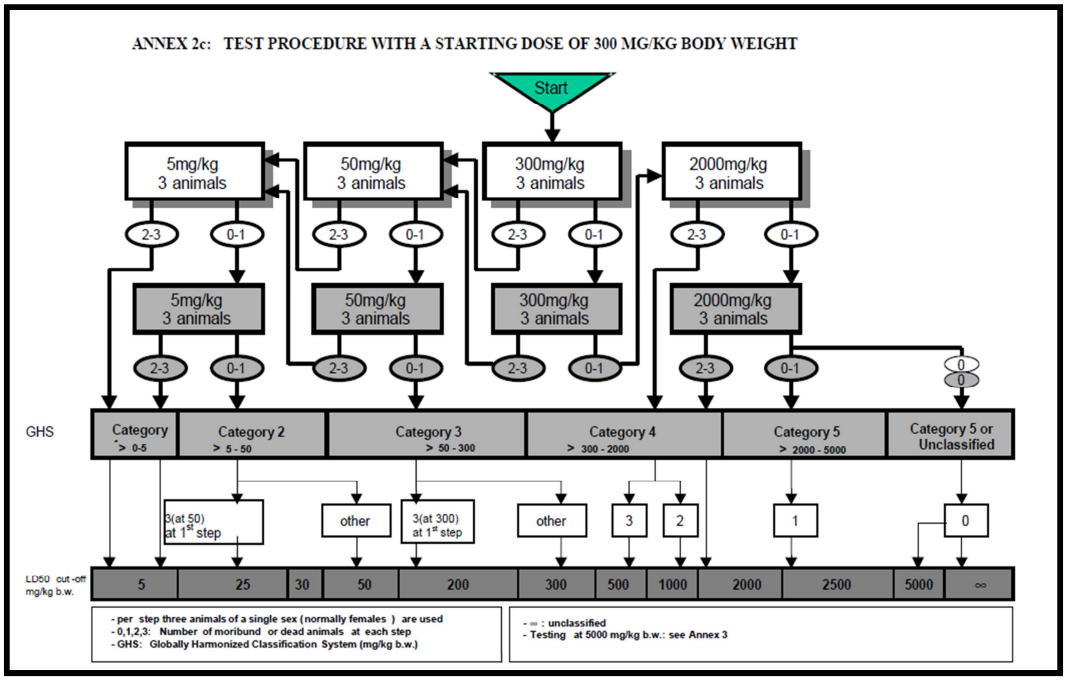
Experimental Design:

The animals for the present study were approved by Institutional Animal Ethical Committee (IAEC) of SA-FORD, proposal no. SA-FORD_FEB_2012. Wistar rats weighing 150-200g were housed in Polycarbonate cages and were maintained at 23° ± 2° C, 30 to 70% RH, 12:12 light/dark cycle. Three animals were used for each step (Figure 1). The starting dose level was selected as 300 mg/kg body weight as no information was available to conduct the study.

The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose was delayed until confidence of survival of the previously dosed animals is attained.

The maximum dose volume administered was 10 ml/kg body weight. 3 rats of the first group G1 were dosed with starting dose of 300 mg/kg body weight and the animals did not show any mortality. So another 3 animals of the same group G1 were dosed with 300 mg/kg body weight and no mortality was observed. Next, 3 animals of group G2 were dosed with 2000 mg/kg body weight and no mortality was observed. So, another 3 animals of the same group G2 were dosed with 2000 mg/kg body weight and no mortality was observed. Hence, further dosing was stopped.Animal nos. 01 to 03 were dosed between 10.56 to 10.57 a.m. Animal nos. 04 to 06 were dosed between 10.47 to 10.48 a.m. Animal nos. 07 to 09 were dosed between 11:20 to 11:24 a.m. Animal nos. 10 to 12 were dosed between 10.04 to 10.05 a.m.

Figure: 1



OBSERVATION

After administration of Multi-herbal formulation ECD0058, individual animals were frequently observed at 30 minutes, 1, 2, 3 and 4 hours post dosing on day 0 (day of dosing) for clinical signs and symptoms. Subsequently, all the animals were observed once a day during the 14 day observation period. At dosing of 300 and 2000 mg/kg body weight, all the animals were observed normal throughout the experimental period (**refer table 2 & 3**).

All surviving animals were observed twice daily (morning and evening) for morbidity and mortality, throughout the acclimatization and study period. No mortality was observed in animals treated with 300 and 2000 mg/kg body weight throughout the 14 days observation period (**refer table 4**).

All the rats were weighed on days 0 (prior to dosing), 7 and 14. Mean body weight of all the animals treated with 300 and 2000 mg/kg body weight were observed with gain on day 7 and 14, as compared to day 0 (**refer table 5 and 6**).

At the end of 14 day observation period, all the rats were euthanised by overdose of CO_2. All the animals were observed for external and internal gross pathology. During external and internal gross necropsy examination, terminally sacrificed animals treated with 300 and 2000 mg/kg body weight showed no abnormalities (**refer table 7**).

Table 2: Individual Animal Clinical Signs and Symptoms on Day '0'

Animal No.	Group/ Dose (mg/kg body weight)	Hours (Day 0)				
		1/2	1	2	3	4
01	G1/ 300	Normal	Normal	Normal	Normal	Normal
02		Normal	Normal	Normal	Normal	Normal
03		Normal	Normal	Normal	Normal	Normal
04		Normal	Normal	Normal	Normal	Normal
05		Normal	Normal	Normal	Normal	Normal
06		Normal	Normal	Normal	Normal	Normal
07	G2/ 2000	Normal	Normal	Normal	Normal	Normal
08		Normal	Normal	Normal	Normal	Normal
09		Normal	Normal	Normal	Normal	Normal
10		Normal	Normal	Normal	Normal	Normal
11		Normal	Normal	Normal	Normal	Normal
12		Normal	Normal	Normal	Normal	Normal

Table 3: Individual Animal Clinical Signs and Symptoms (Continued)

Animal No.	Group/ Dose (mg/kg body weight)	Days post dosing													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
01	G1/ 300	Normal													
02		Normal													
03		Normal													
04		Normal													
05		Normal													
06		Normal													
07	G2/ 2000	Normal													
08		Normal													
09		Normal													
10		Normal													
11		Normal													
12		Normal													

Table 4: Individual Animal Mortality Record

Animal No.	Group/ Dose (mg/kg body weight)	Day of Observation (Day 0 to 14)	
		Morning Observation	Evening Observation
01	G1/ 300	No mortality and morbidity	No mortality and morbidity
02		No mortality and morbidity	No mortality and morbidity
03		No mortality and morbidity	No mortality and morbidity
04		No mortality and morbidity	No mortality and morbidity
05		No mortality and morbidity	No mortality and morbidity
06		No mortality and morbidity	No mortality and morbidity
07	G2/ 2000	No mortality and morbidity	No mortality and morbidity
08		No mortality and morbidity	No mortality and morbidity
09		No mortality and morbidity	No mortality and morbidity
10		No mortality and morbidity	No mortality and morbidity
11		No mortality and morbidity	No mortality and morbidity
12		No mortality and morbidity	No mortality and morbidity

Table 5: Individual Animal Body Weight (g) and Body Weight Changes (%)

Animal No.	Group/ Dose (mg/kg body weight)	Dose Volume* (ml)	Body Weight (gram)			Body Weight Change (%)	
			Day 0	Day 7	Day 14	Day 0-7	Day 0-14
01	G1/ 300	1.5	145	163	198	12.41	36.55
02		1.5	150	168	200	12.00	33.33
03		1.4	144	159	186	10.42	29.17
04		1.3	134	159	182	18.66	35.82
05		1.4	140	167	173	19.29	23.57
06		1.3	132	155	181	17.42	37.12
07	G2/ 2000	1.2	120	143	192	19.17	60.00
08		1.4	142	177	210	24.65	47.89
09		1.4	139	168	200	20.86	43.88
10		1.4	136	156	178	14.71	30.88
11		1.4	142	162	194	14.08	36.62
12		1.4	139	155	171	11.51	23.02

*= Dose volume calculated based on day 0 body weight

Table 6: Summary of Animal Body Weight (g) and Body Weight Changes (%)

Group/ Dose (mg/kg body weight)		Rats Body Weight (g)			Body Weight Changes (%)	
		Day 0	Day 7	Day 14	0-7	0-14
G1/ 300	Mean	140.83	161.83	186.67	15.03	32.59
	SD	6.88	5.08	10.46	3.85	5.30
	n	6	6	6	6	6
G2/ 2000	Mean	136.33	160.17	190.83	17.50	40.38
	SD	8.31	11.72	14.29	4.91	13.11
	n	6	6	6	6	6

Keys: SD = Standard Deviation, n = Number of Animals

Table 7: Gross Necropsy Observation

Animal No.	Group/ Dose (mg/kg body weight)	Mode of Death	Gross Observation	
			External	Internal
01	G1/ 300	TS	No abnormality detected	No abnormality detected
02		TS	No abnormality detected	No abnormality detected
03		TS	No abnormality detected	No abnormality detected
04		TS	No abnormality detected	No abnormality detected
05		TS	No abnormality detected	No abnormality detected
06		TS	No abnormality detected	No abnormality detected
07	G2/ 2000	TS	No abnormality detected	No abnormality detected
08		TS	No abnormality detected	No abnormality detected
09		TS	No abnormality detected	No abnormality detected
10		TS	No abnormality detected	No abnormality detected
11		TS	No abnormality detected	No abnormality detected
12		TS	No abnormality detected	No abnormality detected

Key : TS = Terminal Sacrifice

RESULT AND CONCLUSION

From the present animal experiment performed as per the OECD Guidelines 423, the results indicate that the Multi-herbal formulation ECD0058 have been found to be non-toxic at 2000 mg/kg body. All the animals did not show any signs of intoxication immediately following dosing and during the observation period of 14 days.

As all the animals survived by the end of the study and gross necropsy did not reveal any major findings, The LD_{50} of Multi-herbal formulation ECD0058 is greater than 2000mg/kg (Category 5 as per OECD guidelines 423 for acute Toxicity Studies) and hence the product ECD0058 is completely safe and does not have any adverse effect on animals.

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