EVALUATION OF ORAL ACUTE TOXICITY OF ETHANOLIC EXTRACT OF *ROSA DAMASCENA* PETALS AS PER OECD GUIDELINES 423

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ABSTRACT

Objective: The present study was designed to carry out acute oral toxicity of find out *Rosa damascena* petals as per Section 4 of Organization for Economic Cooperation and Development (OECD) 423 guideline

Methods: Female Mice was administered ethanolic petal extract of *Rosa damascena* in a single dose by oral gavage at the volume of 1ml/100g of animal at the dose levels of 5, 50, 300, 2000 mg/kg body weight. Mice are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for an overall of 14 days for mortality, wellness parameters and body weight.

Results: The highest dose administered (2000 mg/kg body weight) did not produce mortality or changes in body weight and wellness parameters of the mice.

Conclusion: The ethanolic petal extract of *Rosa damascena* is classified in class 5 (substance with LD_{50} higher than 2000 mg kg-1 and less than 5000 mg kg-1) and is being considered as having low toxicity.

Key Words: *Rosa damascena*; Acute Oral Toxicity; OECD Guidelines 423; Lethality (LD_{50}).

1. INTRODUCTION:

OECD Guidelines for the Testing of Chemicals are a set of internationally recognized specifications for the testing of chemicals decided on by the Organisation for Economic Co-operation and Development (OECD). Section 4 of OECD primarily deals with Health Effects and an OECD 423 guideline in this section is used to evaluate Acute Oral toxicity with the help of Acute Toxic Class Method. The method allows a substance to be ranked and classified according to the Globally Harmonised System for the classification of chemicals which cause acute toxicity by using predefined doses. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100% (OECD, 2002).

Rosa damascena, known as Damask rose, a perennial bushy shrub, is the most famous ornamental plant and the holy ancient herb with novel applications belonging to the Rosaceae family. Its vernacular names include Gulab in Hindi, Gulaabi poovvu in Telugu and Mahakumari or Satapatri in Sanskrit. Chemical composition revealed the presence of Citronellol, geraniol, nerol, phenyl ethyl alcohol; nonadecane, nonadecene, eicosane, heneicosane, tricosane, a-guaiene, geranyl acetate, and eugenol have been reported (Mohaddese, 2016). The most beneficial effects of *R. damascena* in ancient medicine are including treatment of abdominal and chest pain, strengthening of the heart, treatment of menstrual bleeding and digestive problems, and reduction of inflammation, especially of the neck, cough remedy for children, gentle laxative as well as Rose oil heals depression, grief, nervous stress, tension, allergies, headaches, and migraine. Some of the proved pharmacological properties include anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and relaxant effect on tracheal (Mohammad et al., 2011). Apart from these gels of *Rosa damascena has been proved for* UV Protective activity (Patil et al., 2011).

The present study was designed to find out LD50 and to ascertain the safety of ethanol extracts of *Rosa damascena* petals by acute oral toxicity study in mice as per Organization for Economic Cooperation and Development (OECD) guideline 425.

2. MATERIALS AND METHODS:

2.1 Collection and authentication of plant material: The fresh flowers of the deciduous shrub of *Rosa damascena* were collected in bulk from the local area of Warangal, Telangana, India. The flowers were authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana. The petals were separated from the sepals and shadow dried.

2.2 Preparation of extract of Rosa damascena petals: The dried powder material (100 g) of the *Rosa damascena* petals was powdered and passed through sieve no.16. This powder material was macerated with water, ethanol, chloroform, ethyl acetate and petroleum ether for 7 days with occasional shaking. The extracts were filtered through muslin cloth, then the filtrate was evaporated under reduced pressure and vacuum dried. The different dose levels of plant extracts were prepared in suitable vehicle and were used for studies.

2.3 Target animal: All experiments and protocols described in the study were approved by the Institutional Animal Ethical Committee (IAEC) of Jayamukhi College of Pharmacy, Warangal. The study was performed using Swiss female mice , nulliparous, non-pregnant, and weighing 25-30 g. Female rats were selected since literature surveys of conventional LD50 tests demonstrate that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive. Animals were housed in polypropylene cages with not more than three animals per cage and maintained under standard condition (12 hours light / dark cycle; temperature 25± 3 ^{0} C; relative humidity 55 ± 5%) and had free access to standard pellet feed (Hindustan Lever Ltd., India) and water *ad libitum*. Clean paddy husk bedding was provided to the mice.

2.4 Methodology: Two types of acute oral toxicity tests, i.e., limit test and main test were performed according to Paragraph 22 of OECD guideline 423. 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 should be 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.

2.4.1 Preparation of animals: All the animals were accustomed to laboratory condition for 1 week before the commencement of experiment.

2.4.2 Mode of administration: The test substance was administered in a single dose by oral gavage using specially designed mice oral needle. Animals were fasted 3 hours prior to dosing (only food was withheld for 3 h but not water)

2.4.3 Preparation of doses: The volume given did not exceed more than 1 ml/100 g body weight. Following the period of fasting, the fasted body weight of each animal was determined, and the dose was calculated according to the body weight. After the substance has been administered, food was withheld for 1-2 hours in mice.

2.4.4 Number of animals and dose levels: According to Paragraph 18 of OECD guideline 423 three animals were used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. Paragraph 19 of OECD guideline 423 suggests that when available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight. Paragraph 20 of OECD guideline 423 suggests that the time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. Paragraph 21 of OECD guideline 423 suggests that exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered.

2.4.5 Observation period: Mice are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a overall of 14 days (Sailesh and Abhilasha, 2015).All the animals were observed at least twice everyday with the purpose of recording any symptoms of ill-health or behavioral changes. Direct observation parameters include tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, and autonomic and central nervous systems, somato-motor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded (Kumar et al., 2017).

3. RESULTS:

The present study conducted as per the OECD guidelines 423 revealed that the ethanolic petal extract of *Rosa damascena* did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. Table 1 indicates the parameters observed before and after the administration of the test substance.

Table 1: Effects of ethanolic extract of *Rosa damascena* on acute oral toxicity test in mice.

Parameters	Observations	5 mg/ kg		50 mg/ kg		300 mg/ kg		2000 mg/ kg	
		Before	After	Before	After	Before	After	Before	After
Physical parameters	Body temperature	N	N	N	N	N	N	N	N
	Skin colour	N	N	N	N	N	N	N	N
	Fur colour	N	N	N	N	N	N	N	N
	Eyes colour	N	N	N	N	N	N	N	N
	Urine colour	N	N	N	N	N	N	N	N
Behavioral effects	Alertness	N	N	N	N	N	N	N	N
	Grooming	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Restlessness	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Irritability	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Reactivity (environment)	N	N	N	N	N	N	N	N
	Eyes opened/closed	N	N	N	N	N	N	N	N
	Tremors	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Twitches	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Convulsions	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Sedation	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Catatonia	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Sleep	N	N	N	N	N	N	N	N
	Ataxia	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
Autonomic effects	Defecation	N	N	N	N	N	N	N	N
	Lacrimation	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Urination	N	N	N	N	N	N	N	N
	Salivation	N	N	N	N	N	N	N	N
	Piloerection	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Mydriasis	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Miosis	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Emesis	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Diarrhea	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve

Sensory responses	Touch	N	N	N	N	N	N	N	N
	Pain	N	N	N	N	N	N	N	N
Reflexes	Pinna	N	N	N	N	N	N	N	N
	Corneal	N	N	N	N	N	N	N	N
Respiratory effects	Apnea	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Dyspnea	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
Somatomotor effects	Abnormal gait	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Righting reflex	N	N	N	N	N	N	N	N
	Body position	N	N	N	N	N	N	N	N
	Limb position	N	N	N	N	N	N	N	N

N-Normal; -ve- Negative/ Absent;

4. DISCUSSION:

Globally, herbal medicine from medicinal plants has been considered an important alternative to modern allopathic medicine particularly in developing countries. Although the herbal medicines are very popular in the society, only few medicinal herbs have been scientifically evaluated for their potential in medical treatment (Bhatt, 2016). However, these bioactive products from medicinal plants are recognized to be safe without any compromising health effect, and thus extensively used as self-medication. The safety of herbal medicines remains a major concern. However, there is a need to establish the studies scientifically on the toxicity and adverse effect of these medicines. Therefore, additional acute oral toxicity study is crucially needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation. It is also a useful parameter to investigating the therapeutic index of drugs and xenobiotics (Gissi, 2017).

From the experiment performed as per the OECD Guidelines 423, the results reveal that the ethanolic extract of *Rosa damascena* petals have been found non-toxic at 2000 mg/kg body weight in experimental animals. Physical observations, Behavioral effects, Autonomic effects, Sensory responses, Reflexes, Respiratory effects and Somatomotor effects were found to be normal. No untoward or adverse effects were noted in the test animals from the doses of 5, 50, 300 or 2000 mg/kg body weight.

5. CONCLUSION:

The ethanolic extract of *Rosa damascena* petals was found to be safe at 2000mg/kg body weight by Oral route. Even after 14 days, mice were found to be well tolerated with no mortality and no signs of toxicity. The extract are classified in class 5 (substance with LD50 higher than 2000 mg/ kg and less than 5000 mg/ kg) and are being considered as having low toxicity.

6. CONFLICT OF INTEREST:

Authors have declared that no conflict of interests exists.

7. ACKNOWLEDGMENT:

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