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DETERMINATION OF MEDIUM LETHAL DOSE (LD50 VALUE) FOR ORAL ACUTE TOXICITY OF ROYAL JELLY

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ABSTRACT

Objective: RJ a honey bee product has a high medicinal values to treat various diseases such as cancer, inflammation, various skin problems etc. current study was designed for the determination of oral medium lethal dose (LD50 value) of RJ. **Material and Method:** The study was directed according to OECD 423 guidelines by using male Swiss albino mice weighing 20-25 gms. all were divided into six groups. Group G1 was served as control group which received normal saline (NS) while group G2 to G6 were orally treated with single daily dose of 100, 500, 1000, 2000 and 5000 mg/kg of RJ, respectively. All the animals were closely observed for any sign of toxicity illness and behavioral changes. **Results:** Obtained results demonstrated that no mortality was observed in all treatment groups for post 24 hours but the dose 2000mg/kg show mortality at 14th day while high dose up to 5000 mg/kg exhibited mortality at day 7 and 14 days observation duration. **Conclusion:** In conclusion, acute exposure of RJ up to 2000 mg/kg was safe in male mice without causing any adverse effects or mortality. The oral LD50 of Rj suggested to be greater than 1616 mg/kg in male mice.

KEYWORDS: Royal Jelly, LD50 Value, Acute Oral Toxicity, Swiss Albino Mice, Mortality.

INTRODUCTION

Royal Jelly (RJ) is a white-yellowish glandular secretion with a slight phenolic smell. Produced from the hypo pharyngeal and mandibular salivary glands of young nurse[1] for all bee larvae, including larvae which are selected to develop into queens are totally fed with RJ until the fifth day of larval life, and for the whole lifetime for the queen bee RJ remains a devoted feed. Moreover, RJ also has a major effect on the lifespan of queen bee. A worker bee lives around 45 days, while a queen bee could live up to five years during which is able to spawn in a day the equivalent of her weight in eggs. Nearly 2000–3000 eggs per day for several years. Numerous studies have focused on the various biological and pharmacological activities of RJ, including its antimicrobial, anti-inflammatory, anti-diabetic, anti-hypertension, anti-oxidant, anti-tumor, anti-aging, and immunoregulatory activities.[2-5]

The acute toxicity testing are conducted to obtain information regarding biological activity of a substance to increase understanding of its mechanism of action. The obtained information by acute systemic toxicity test is used in hazard identification and risk management in the context of production, handling, and use of chemicals. The LD50 value, in an acute toxicity test is defined as the statistically derived dose, when administered, is expected to cause death in 50% of the treated animals in a set period, is currently the basis for toxicological classification of chemicals.[6] Often both sexes of Laboratory mice and rats are generally used for a typical LD50 study. Acute toxicity studies are directed to estimate the effects of a single substance, thus commonly each animal receives a single dose of the test substance. Depending upon the survival rate in the initial group, additional groups are added for the study at higher and/or lower doses. Survival is evaluated at fixed interval after dosing, generally 7 or 14 days, but infrequently as early as 24 hr. Literature search showed that no toxicity study has been conducted on RJ in animals or humans. Thus, the present study is designed to determine the LD50 value of RJ in mice which provides preliminary data to other researchers to elicit the safe and appropriate safe doses for pre-clinical study.

MATERIALS AND METHODS

Collection and preparation of royal jelly suspension: RJ was purchase from the apiculture farm of Hi-tech Natural product (India) Ltd. From colonies of *Apis mellifera* in the lyophilized form. Different concentration of royal jelly were dissolved in 5% CMC (Carboxy methyl cellulose).

Animals: Inbred strains of Swiss albino male mice (20 – 25 g) were obtained from the Wockhardt research institute Aurangabad Maharashtra India. Based on the following properties a particular species selected.

Animals are biologically similar to humans. Usually Albino rats and mice are preferred for In-vivo study because, Mice shares more than 98% DNA with humans, therefore these are susceptible to numerous same health problems as humans. Mice have shorter life cycle than humans therefore they can be studied all over their whole life span.

The mice were housed in standard polypropylene cages, under standard laboratory conditions (25 ± 1°C temperature; 12:12 h light/dark and 50-60 % humidity) Standard rodent chow diet (VRK Nutritional solution, Pune, Maharashtra, India.) and water were provided ad libitum to them. They were isolated for 7 days prior to the start of the study. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy, Aurangabad, and (MH) India. (Ref. No. CPCSEA/IAEC/Pcology-53/2017-18/134.)

Experimental design for acute toxicity studies

Acute toxicity studies were performed for RJ according to the toxic classic method following guidelines 423 prescribed by OECD.[7] Overnight the animals were kept fasting and only water is given to them. These were divided into five groups (G1-G5) each containing six animals. G1: kept as control group and 1.0 ml of physiological saline was given. G2-G5: Each of these groups was then administered with royal jelly as 5, 50, 300 and 2000 mg/kg orally. After administration of the first dose, the animals were observed continuously for 30 min and then periodically for first 24 h with special attention during the first 4 h and afterwards daily for a total of 14 days. All observations (sedation, convulsions, tremors, salivation, lethargy, death etc.,) are scientifically noted with individually records of each animal. By using the arithmetic method of Karber as modified by Aliu and Nwude, The LD50 of the royal jelly was calculated.[8]

Table. 1: Determination of LD50 of Royal jelly in mice.

Sr. No.	Dose of Rj (mg/kg)	No of Animals exposed	No of deaths at intervals						Dose difference (a)	Mean mortality (b)	Y (a x b)
			24 h	48 h	72 h	96 h	7day	14 day			
1	1 ml (NS)	3	-	-	-	-	-	-	-	0	0
2	5	3	-	-	-	-	-	-	5	0	5
3	50	3	-	-	-	-	-	-	45	0	45
4	300	3	-	-	-	-	-	-	250	0	250
5	2000	3	-	-	-	-	-	1	1700	0.5	850
										Sum	1150

The LD50 was calculated using the formula:

LD50 = LDy – Σ (ax b)/N

Where LDy =Maximum dose

N =Number of animals per group

a =Dose difference

b =Mean mortality test animals

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RESULTS AND DISCUSSION

The symptoms and signs of toxicities were observed in skin, fur, eye membranes, respiratory, behavior patterns etc.[9] after administration of first dose of each concentration, during the first 24 hours, all mice showed no symptoms of toxicity. 2000mg/kg at day 14 shows aggressive behavior, loss of appetite and obvious signs of passive behavior as compared with control mice.

Table 1 exhibited the results of the acute oral toxicity of Royal jelly in mice from day one to day 14. All the male mice that received 5, 50, 300 and 2000 mg/kg of RJ did not show any toxic signs and abnormal behavioral changes and mortality post 24 hours of RJ treatment. The dose 2000mg/kg show mortality at 14th day (0.5) compared to all other groups. (Table 1).Thus according to the OECD guidelines 423 it should be in the category 5. That is not classified as a toxic and demonstrated LD50 value of RJ to be 2500 mg/kg. But according to the method of Karber as modified by Aliu and Nwude the calculated LD50 value for royal jelly is 1616 mg/kg B. W.

Acute toxicity study could be able to provide important information to identify the targeted organs of test substances after acute exposure.[10] The result demonstrated that acute oral toxicity (LD50) of RJ was found to be 2500 mg/kg (category 5). Thus on the basis of obtained result RJ dose 100, 250 and 500 mg/kg were selected for chronic treatment. Because the dose up to 2500mg/kg, Based on the classification of chemical toxicity as described in the OECD 423 guideline, the toxic profile of RJ is classified as closely to category 5 which is low acute toxicity hazard.[7] This has been the first study conducted on the acute oral toxicity of RJ for the LD50 determination in mice and these findings are very crucial for choosing the therapeutic dose in clinical trials. Presently, the chemical labeling and classification of acute systemic toxicity based on oral LD50 values recommended by the Organization for Economic Co-operation and Development(OECD) are as follow: category 1: very toxic >0-5 mg/kg, category 2: toxic >5-50, category 3: harmful >50-300mg/kg, category 4: no label >300-2000 mg/kg, category 5: >2000-5000 mg/kg.

Current experiment exhibited from the dose of 2000mg/kg, lethal effect started manifesting. Consequently the LD50 of 1616 mg/kg of RJ indicated that RJ has no adverse effect up to this dose, ever since none has been observed in the past. Many variables such as animals' species and strain, age, gender, diet, bedding, temperature, caging conditions and time of the day, all can affect the obtained LD50 value thus, it has not been regarded as a biological

constant. Hence there are significant doubts in concluding LD50 value for a specie to other species. As a result, spotting LD50 test is only a rough estimate of human lethality.[11] Derelanko has postulated the inter-species dose conversion based on the equivalency of surface area. The conversion factor of mouse (20g) to human (60 kg) is 1/12.[12] Thus, the 100mg/kg of RJ in mice is equivalent to 8.3 mg/kg, 250mg/kg of RJ in mice is equivalent to 20.8 mg/kg and 500mg/kg of RJ in mice is equivalent to 41.6 mg/kg in humans.

However, it is too early to conclude the safety of RJ since it has been studied only in experimental animals but at least it provides some correlation between information obtained from animal study to human usage. Many interspecies factors such as different expression of enzymes, metabolic rates and physiological changes between mice and humans need to be carried out to confirm our suggestions. Further evaluation of the sub-chronic oral toxic effect in mice and rats needs to be carried out for better understanding about the mechanisms of RJ.

CONCLUSION

Single oral administration of RJ up to 1000mg/kg did not cause any mortality and adverse effects in mice up to 14 days. According to OECD, royal jelly is of category 5 compound i.e. non- toxic. A single dose of 100, 250 and 500 mg/kg of RJ was safe to mice and the LD50 value of RJ was greater than 1616 mg/kg BW in mice.

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