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TOXICOLOGICAL EVALUATION OF *CALENDULA OFFICINALIS* - LINN

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The aqueous extract of *Calendula officinalis* - Linn (flowers, roots and whole plant) was evaluated for oral as well as intravenous toxicity in rats and mice and LD_{100}, LD_{50}, ED and TH was determined. The toxicity was also found to be dependent on the dose and route of administration.

Key words : *Calendula officinalis*, Toxicity, Lethal dose.

Introduction

Calendula officinalis - Linn belongs to the natural order compositeae. It is an annual herb, popularly known as "Marigold or Genda". It is cultivated all over the world as an ornamental garden plant [1-3].

Marigold has a long history of medicinal use not only in eastern system of treatment but in homoeopathic system of treatment [4,5] also. Systematically/internally marigold is used as a remedy for epileptic fits, fever, kidney troubles [2], muscular pains, as sedative, hypotensive [6] in bleeding piles [2], in cancer chemotherapy [7,8], as a hypocholesterimic agent [9] and in ulcers [2,10]. It also has an astringent [1,3], anti- inflammatory [11,12] antimicrobial [13-15] and haemostatic [16] action.

The flowers are used as a source of Provitamin A [17]. Commercially the flowers are used as a dye substitute for Analto [18] and as a colour additive [19]. The oil obtained from the seeds are used in soaps, cosmetics and perfumaries [20].

An ample data regarding the chemical composition of this plant is available in literature which reveals the presence of terpenes, triterpenes, glycosides, sterols, alkaloids, tannins, salicylic acid, flavonoids, pigments, carotenoids, nicronic acid, phytosterin, phenols, essential oils, resins and eighteen n- paraffin [4,5,21].

The activities and contributions of marigold are many and varied. Therefore, to ensure safety of use, toxicological evaluation is necessary because it will define the limits of safe use of marigold, which will be more than parochial and will quantify the risk of untowards signs and symptoms according to dose.

Materials and Methods

Fully grown mature plants cultivated in PCSIR Laboratories Complex, Karachi were removed from their beds, washed and dried in air. Flowers, leaves and whole plant (1 kg each) were chopped into small bits and soaked in 95% ethyl alcohol (5 litres) for 96 hr. The solvent was decanted and concentrated in vacue. The resulting gel like mass was partitioned between water and petroleum-ether (2:1 v/v). Aqueous layer was then separated and concentrated under reduced pressure at room temperature into a semi-solid mass. This semi-solid mass was used for further studies and was referred as aqueous part.

Toxicity studies. Healthy albino rats and mice (male and female), reared at PCSIR Animal House, weighing 100-120 gms and 25-30 gms respectively were selected for oral as well as for parenteral (intravenous) toxicity test. Animals were kept in optimal experimental condition and were observed for a period of 7 days before use.

Animals used for testing were housed in plastic cages with sliding perforated stainless steel covers. The dimension of the cages were 12.0 x 8.5 inches at top 10.5 x 8.0 inches at bottom and 6.5 inches high. Normal routine feed was given to animals. Water was supplied freely by means of inverted bottles which were placed on top of stainless steel covers. To facilitate the movement of rats and mice, saw dust was spread on the floor of cages. Cages were marked with their respective doses. Each dose was repeated thrice to confirm the results.

Oral toxicity. The drug was fed orally by means of appropriate feeding canula in a dose of 500 to 6000 mg/kg body weight, keeping the volume constant. Care was taken not to injure the animal while feeding and were observed for a period of 7 days.

Parenteral (intravenous) route. The intravenous toxicity was done by injecting the drug through tail vein in different doses. The total volume of each intravenous injection was kept constant to avoid volume variation effects. Animals were observed for a period of 7 days after injecting the drug.

Results and Discussion

Assessment of toxicological manifestation of three different extracts i.e. flowers, leaves and whole plant was done on rats and mice by oral as well as by intravenous route. The nature of signs and symptoms observed in both species were found to be the same. Marked variation in the severity and depth of symptoms were proportional to the concentration of the drug (aqueous extract) used and the route of administration.

Oral toxicity. No mortality was observed in rats upto a dose of 6000 mg/kg during the said observation period. A dose upto 1500 mg/kg by oral route caused vasodilatation, activeness and alertness in animals, while a dose above that caused vasoconstriction leading to pallor in eyes and external ears which clearly reflected that the action of the aqueous extract of marigold (flowers, leaves and whole plant) is dose-dependent.

Other manifestations are pupillary dilatation, marked pilo-erection, muscles spasms, hyper-irritability, lacrimation, tremor, increased heart rate (tachycardia), dryness of mouth, absence of salivation, causing increased thirst, hyperpnea, loss of righting reflexes, hiccough and frequent urination. As the time passed on, the animal was unable to maintain the normal posture of the body. This induction time ranges from 5-45 min depending upon the concentration of the drug used.

Intravenous toxicity. Acute toxicity test was carried out on rats and mice to evaluate any untowards effect on the body and to establish ED (effective dose), LD_{50} (lethal dose which kills 50% of experimental animals) and LD_{100} (lethal dose that kills 100% of experimental animals). ED, LD_{50} and LD_{100} for rats and mice are given in Tables 1 and 2.

No mortality was observed upto a dose of 760, 700 and 710 mg/kg in rats and 52, 55 and 54 mg/100 gm in mice for flowers, leaves and whole plant and was taken as ED (effective dose). The dose was then increased gradually by increasing 10 mg each time in case of rats and 1 mg in case of mice. The increase in the concentration of drug resulted in mortality, until a dose was obtained which killed 50% of the experimental animals i.e. LD_{50}. The LD_{50} for flowers, leaves and whole plant in rats was 800, 780, 790 mg/kg for mice; it was 58, 60, 62 mg/100 gm whereas LD_{100} was 1000, 950, 950 mg/kg for rats; 65, 68, 68 mg/100 gm for mice respectively for flowers, leaves and whole plant. Mortality was observed at a dose of 770, 720, 720 mg/kg for rats; 53, 56 and 55 mg/100 gm for mice respectively. This was termed as TH (threshold of the drug).

Signs and symptoms observed were same as observed in the oral route. The difference was only in the time of onset and duration of action. Severity of signs and symptoms and duration of action gave clear indication that signs and symptoms were based not only on dose but also on the route of administration. By intravenous route the reaction was quick and took no time to show the symptoms due to the rapid systemic distribution of test material throughout the animal body in a short time. This is that period of time, which is taken by the blood to circulate and necessary for translocation of test material from capillaries to extracellular fluid.

The important features observed were ataxia (abnormal gait, disturbed equilibrium), hemiparaplegia (paralysis of only one hind limb) with marked depression and mental cloudiness. Animal remains in this condition for 5-35 min.

In doses as shown in Table 1 and 2 where mortality was observed, it was noticed that cessation of respiration followed by asystole was a major cause of death. In cases where animals survived the drug elevated the pain threshold and concomitantly decreased awareness and increased the ability to accept noxious stimuli without the arousal of anxiety and fear of suffering.

TABLE 1. INTRAVENOUS TOXICITY OF AQUEOUS EXTRACT OF *CALENDULA OFFICINALIS* IN RATS.

		Flowers							Leaves						Whole plant					
Sr No.	No.of animals	Dose in mg/kg	% of survival	% of mortality	ED	TH	LD_{50}	LD_{100}	% of survival	% of mortality	ED	TH	LD_{50}	LD_{100}	% of survival	% of mortality	ED	TH	LD_{50}	LD_{100}
1.	10	500	100	0	-	-	-	-	100	0	-	-	-	-	100	0	-	-	-	-
2.	10	600	100	0	-	-	-	-	100	0	-	-	-	-	100	0	-	-	-	-
3.	10	700	100	0	-	-	-	-	100	0	ED	-	-	-	100	0	-	-	-	-
4.	10	710	100	0	-	-	-	-	90	10	-	-	-	-	100	0	ED	-	-	-
5.	10	720	100	0	-	-	-	-	90	10	-	TH	-	-	90	10	-	TH	-	-
6.	10	730	100	0	-	-	-	-	70	30	-	-	-	-	80	20	-	-	-	-
7.	10	740	100	0	-	-	-	-	60	40	-	-	-	-	70	30	-	-	-	-
8.	10	750	100	0	-	-	-	-	70	30	-	-	-	-	70	30	-	-	-	-
9.	10	760	100	0	ED	-	-	-	60	40	-	-	-	-	70	30	-	-	-	-
10.	10	770	90	10	-	TH	-	-	60	40	-	-	-	-	60	40	-	-	-	-
11.	10	780	70	30	-	-	-	-	50	50	-	-	LD_{50}	-	60	40	-	-	-	-
12.	10	790	70	30	-	-	-	-	40	60	-	-	-	-	50	50	-	-	LD_{50}	-
13.	10	800	50	50	-	-	LD_{50}	-	30	70	-	-	-	-	30	70	-	-	-	-
14.	10	850	40	60	-	-	-	-	30	70	-	-	-	-	30	70	-	-	-	-
15.	10	900	30	70	-	-	-	-	10	90	-	-	-	-	10	90	-	-	-	-
16.	10	950	10	90	-	-	-	-	0	100	-	-	-	LD_{100}	0	100	-	-	-	LD_{100}
17.	10	1000	0	100	-	-	-	LD_{100}	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2. INTRAVENOUS TOXICITY OF AQUEOUS EXTRACT OF *CALENDULA OFFICINALIS* IN MICE.

			Flowers						Leaves						Whole plant					
Sr No.	No.of animals	Dose in mg/100g (m/g)	% of Survival	% of mortality	ED	TH	LD_{50}	LD_{100}	% of Survival	% of mortality	E.D	TH	LD_{50}	LD_{100}	% of Survival	% of mortality	ED	TH	LD_{50}	LD_{100}
1.	10	50	100	0	-	-	-	-	100	0	-	-	-	-	100	0	-	-	-	-
2.	10	51	100	0	-	-	-	-	100	0	-	-	-	-	100	0	-	-	-	-
3.	10	52	100	0	ED	-	-	-	100	0	-	-	-	-	100	0	-	-	-	-
4.	10	53	90	10	-	TH	-	-	100	0	-	-	-	-	100	0	-	-	-	-
5.	10	54	80	20	-	-	-	-	100	0	-	-	-	-	100	0	ED	-	-	-
6.	10	55	70	30	-	-	-	-	100	0	ED	-	-	-	90	10	-	TH	-	-
7.	10	56	70	30	-	-	-	-	90	10	-	TH	-	-	80	20	-	-	-	-
8.	10	57	60	40	-	-	-	-	80	20	-	-	-	-	80	20	-	-	-	-
9.	10	58	50	50	-	-	LD_{50}	-	70	30	-	-	-	-	70	30	-	-	-	-
10.	10	59	40	60	-	-	-	-	60	40	-	-	-	-	70	30	-	-	-	-
11.	10	60	40	60	-	-	-	-	50	50	-	-	LD_{50}	-	60	40	-	-	-	-
12.	10	61	30	70	-	-	-	-	40	60	-	-	-	-	60	40	-	-	-	-
13.	10	62	20	80	-	-	-	-	40	60	-	-	-	-	50	50	-	-	LD_{50}	-
14.	10	63	10	90	-	-	-	-	30	70	-	-	-	-	40	60	-	-	-	-
15.	10	64	10	90	-	-	-	-	30	70	-	-	-	-	40	60	-	-	-	-
16.	10	65	-	100	-	-	-	LD_{100}	20	80	-	-	-	-	30	70	-	-	-	-
17.	10	66	-	-	-	-	-	-	10	90	-	-	-	-	20	80	-	-	-	-
18.	10	67	-	-	-	-	-	-	10	90	-	-	-	-	10	90	-	-	-	-
19.	10	68	-	-	-	-	-	-	-	100	-	-	-	LD_{100}	0	100	-	-	-	LD_{100}

Conclusion

From the observations made, it can be concluded that the drug in therapeutic doses reduced respiratory minute volume and thus carbondioxide is increasingly retained with increased amount of the extract used resulting in death. This may be due to the higher drug levels which depresses respiration.

Therefore, the aqueous extract of marigold (flowers, leaves, and whole plant) is a centrally acting analgesic and has a marked depression on the cardio-respiratory centres. This specialized type of depressant action upon the central nervous system results in obtunding of pain sensation without the loss of consciousness in therapeutic doses.

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