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Behavioural Effects of Alcoholic Extract of Different Doses of Inula Racemosa On Mice-An Experimental Evaluation

KEYWORDS: Inula racemosa, Behavioural effect, alcoholic extract, Mice

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ABSTRACT *Objective: To evaluate the alcoholic extract of effect of Inula racemosa on behavioural aspects on mice.*

Methods: Behavioural effect of alcoholic extract of Inula racemosa was studied in mice with the help of Digiscan Animal Activity Monitoring system. Albino mice of either sex weight 25-30 gm had been used for this study. These mice were bred and raised in a laboratory. The mice were divided into four groups of six mice per group. Within each group, mice were administered per orally with one of the following incremented dose of drug dissolved in 1% gum acacia w/v solution in 0.9% sterile saline 2 ml/kg vehicle, 60. 120 and 240 mg/kg.

Results: When treated with 60 mg/kg p.o. of alcoholic extract of Inula racemosa, a significant decrease was observed in the horizontal activity at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.05$) minutes and increased at 120 ($p<0.05$) minutes as compared to controls. When treated with 120 mg/kg, a significant decrease was observed in the horizontal activity, the total distance, movement time, sterotype counts, marginal time at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.001$) minutes. When treated with 240 mg/kg p.o. of alcoholic extract of Inula racemosa, a significant decrease was observed in the horizontal activity, total distance, movement time, sterotype counts, marginal time at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.001$) minutes and 120 ($p<0.001$) minutes.

Conclusion: Inula racemosa has only mild sedative effect in therapeutic doses which was the common side effect of classical anti-histaminics.

INTRODUCTION

Millions of plants constitute the floristic treasure on the planet earth and are integral part of human life. They provide food, fodder, shelter, fibre, timber, construction material, furniture, dyes, gums, fuels and many other applications in daily life. Moreover, they are excellent source of various herbal medicines useful in the treatment of various human and animal diseases. Medicinal plants offer alternative remedies for different health problems. Several drugs are available in the market for the different diseases (Rajagopal and Sasikala, 2009; Rao et al, 2001).

Ayurveda is the most ancient health care system of India, Srilanka and other countries (Chopra and Doiphode, 2002). The World Heath Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Every now and then we come across patients of asthma seeking ayurvedic treatment and advice. This is because of the versatile approach of ayurveda to the root cause of the problem and its belief in preventing the disease rather than treating it. Inula racemosa is highly valued in "Indian System of medicine" possessing potential role in allergic asthma and related respiratory affliction as reported. Inula racemosa (IR) is an ornametal plant of the asteraceae family. It grows in the temperate and alpine western Himalayas, and it is common in Kashmir., commonly known as puskarmula, it is well known herb in India for its medicinal properties. The roots of puskarmula are used for the medicinal purpose.

Much of the investigations support the therapeutic efficacy of the plant as antihyperglycemic (Gholap and Kar, 2003), cardiac activity (Lokhande et al, 2006) and insecticidal and phytotoxic (Liu et al, 2006) etc. as well plant is a major ingredients in many ayurvedic formulations.

Traditional plant based remedies are still the first choice in the developing countries because of their cost effectiveness, easy availability and minimum or no side effects (Kameswara, 1999; Okigbo and Mmeka, 2006).

As the behavioural aspect of the drug is important from undesirable effect and compliance point of view, this study was designed to evaluate the alcoholic extract of effect of Inula racemosa on behavioural aspects on mice.

MATERIAL AND METHODS

Behavioural effect of alcoholic extract of Inula racemosa was studied in mice with the help of Digiscan Animal Activity Monitoring system. Albino mice of either sex weight 25-30 gm had been used for this study. These mice were bred and raised in a laboratory. Animal had continuous access to food and water at all time except while being tested.

All mice were maintained in a temperature controlled room (30°C) with a 12 hr light-dark cycle (0700-1900 hours light on). The pharmacological testing was carried out between 900 to 1300 hours. The mice were divided into four groups of six mice per group. Within each group, mice were administered per orally with one of the following incremented dose of drug dissolved in 1% gum acacia w/v solution in 0.9% sterile saline 2 ml/kg vehicle, 60. 120 and 240 mg/kg. For acclimation, all mice were placed in the infrared detection chamber (42x 42x 30 cm high inside dimension) one hour prior to actual testing.

Assessment of locomotor activity was carried out with the help of Digiscan Animal Activity Monitor; Omnitech Electronics, Colombus, Ohio, USA. This computerised system allowed detailed analysis of many aspects of locomotor behaviour in three dimensional space, including horizontal activity , total distance travel (cm), number of movements,

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movement time (seconds (sc) resting time (sc) vertical activity, number of vertical movements, vertical time (sc), stereotypy counts, no. of stereotypy, stereotypy time (sc), clockwise revolutions, margin time (sc), centre time (sc), time spend in the comer (sc), left front, right front, left rear and right rear by means of infrared photodetectors. The horizontal activity, no. of movement and average speed of movement reflects variable that assess ambulation, whereas vertical activity, vertical time and number of vertical movement are three aspects of rearing behaviour.

The horizontal activity counter of the digiscan records total number of photo beam interruptions, whereas in vertical activity, there are total number of interruptions of vertical sensors. The horizontal (ambulatory) activity counter ignores repetitive interruptions of the same photobeams with 1 second in between, such as grooming, scratching, head bobbing which reflect the stereotypy behaviour.

Digiscan monitors animal locomotor activity via a grid of invisible infrared light beams traverse the animal cage in the X (left-right), Y (front-back) and Z (vertical) axis. Sensor beams are located 2.5 cm above the floor and 2.5 cm apart. Interruption of these beams generated data that were collected by an analyser and determine the animal position 100 times per second. The analyser had effectively develop a dynamic picture of the animal, which revealed whether the animal was resting, ambulating, rearing or performing stereotypic activity and generated results, which were printed automatically at the end of each time period.

The animals were observed for behaviour prior to base line control and after drug treatment every 10 minutes for a duration of 120 minutes. The mice were observed for any significant alteration in behaviour, same animal was used as its own control. Data were expressed as mean percentage change from control value. The horizontal activity, total distance, movement time, stereotypy count and margin time were analysed.

Preparation of Alcoholic Extract:
Roots of Inula racemosa (Pushkar-mool) were purchased from local market and identified by specialist of pharmacognosy. The roots were separated and dried again in shed. The dry powder of roots was prepared with the help of electric grinder. This powder was filled in empty capsules of 500 mg capacity for clinical trial. Same powder was also used for preparation of alcoholic extract.

Five Kilograms of powdered material was extracted with 70% alcohol in Soxhlet apparatus by process of continuous heat extraction at 55^0–60^0 C (Devis 1961). After filteration the extract was concentrated on a water bath under reduced pressure. A semisolid material was left behind which was dried again in a vaccum dessicator over anhydrous calcium chloride. The weight of extract thus obtained was 775 grams, this was preserved in refrigerator. The extract was suspended in normal saline with gum acacia (5% w/v) for ready use, just before the experiment.

Statistical analysis
The results are presented in mean±SE. The paired t-test was used to compare changes in the study group to controls. The p-value<0.05 was considered significant. All the analysis was carried out by using SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

Table-1 shows the behavioural Effects of Alcohlic Extract of Inula racemosa (60 mg/kg) in Mice. When treated with 60 mg/kg p.o. of alcoholic extract of Inula racemosa, a significant decrease was observed in the horizontal activity at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.05$) minutes and increased at 120 ($p<0.05$) minutes as compared to controls. The total distance, movement time, sterotype counts and marginal time were also significantly ($p<0.05$) decreased at 30, 60 and 90 minutes as compared to controls. A significant decrease also noted in movement time, sterotype counts and marginal time at 120 minutes ($p<0.05$) as compared to controls.

When treated with 120 mg/kg p.o. of alcoholic extract of Inula racemosa, a significant decrease was observed in the horizontal activity, the total distance, movement time, sterotype counts, marginal time at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.001$) minutes and 120 ($p<0.001$) minutes except for horizontal activity at 120 minutes which insignificantly increased as compared to controls (Table-2).

When treated with 240 mg/kg p.o. of alcoholic extract of Inula racemosa, a significant decrease was observed in the horizontal activity, The total distance, movement time, sterotype counts, marginal time at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ($p<0.001$) minutes and 120 ($p<0.001$) minutes (Table-3).

DISCUSSION
The interest in medicinal plants and phytochemicals has increased for their therapeutic properties in human diseases, including hepatic system. Inula racemosa root has been demonstrated to relieve ischemic pain and exhibit cardioprotective effect. Sesiquiterpenes, alantolactone, isoalantolactone, alloalantolactone, and essential oil are the major constituents which accounts for bioactivity of this herb. It also contains several flavanol glycosides, germacranolides and eudesmenes etc. This plant may offer new alternatives to the limited therapeutic options that exist at present in the treatment of liver diseases or their symptoms, and they should be considered for future studies. The potent hepatoprotective activities of the chemically defined molecules isolated from natural origins represent an exciting source for effective liver protective agents (Prathyusha et al, 2013).

In an investigation, alcoholic extract of root of Inula racemosa, was studied for its antiallergic effect in experimental models of type I hypersensitivity, viz. egg albumin induced passive cutaneous anaphylaxis (PCA) and mast cell degranulation in albino rats. Inula racemosa (i.p. as well as p.o.) showed significant protection against egg albumin induced PCA. Protection against compound 48/80 induced mast cell degranulation by alcoholic extract of Inula racemosa (single dose) was similar to that of disodium cromoglycate. The seven days drug treatment schedule showed greater protection than disodium cromoglycate intraperitoneally. The results suggest that Inula racemosa possesses potent antiallergic properties in rats (Srivastava et al, 1999).

The behavioural effects of Inula racemosa was analysed in this study. The results of the present study showed that the compound had slight central nervous system (CNS) depressant activity. Graded doses (60, 120 and 240 mg/kg) of alcoholic extract of Inula racemosa produced a dose-dependant decrease in horizontal activity, total distance travelled which denoted a decrease in ambulation behavior. The movement time and stereotype counts were also increased as compound to control group of animals. The

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resting time was also increased, such behavioural change denotes a mild CNS depressant activity. The animal spent maximum time at the central area of the cage and time spent at the marginal areas of the cage were decreased. Besides this, there was no change in the other behavioural parameters. The effect started after 15 minutes and peak effect was observed between 45-60 minutes and the effect lasted up to 2 hours.

In a study, the ischemia/reperfusion injury causes significant increase in the levels of AST, ALT, ALP and LDH in model control group indicating the cell damage and tissue injury whereas supplementation with hydroalcholic extract of Inula racemosa significantly reduced the elevated levels of above parameters. Histopathological analysis showed high degree of congestion and mild necrosis in model control group which was reduced to minimum levels in drug treated groups. Inula racemosa increased the free radicals scavenging activity in the early period of hepatic IR injury in rats (Prathyusha et al, 2013).

Puskaramula works well in pleurisy, even tubercular, by ameliorating the infection, fever, pain and cough. It imparts a stimulant action on the heart and reduces the breathlessness due to cardiac asthma. It possesses a mild diuretic property, hence is used with benefit in dysuria. In ayurveda, it is widely used for various disorders, it is mostly used in heart and respiratory disorders. The rhizome act as antiseptic, anti-bacterial, anti-fungal, anti-inflammatory, analgesic and mild diuretic. It is used in the treatment of contagious fevers, anginapectoris, heart disease and ischemic heart disease. It is also used in cough, hiccup, bronchial asthma,indigestion, flatulence, inanorexiaandinfever (Patel et al, 1982; Tripathi et al, 1984).

Thus, Inula racemosa has only mild sedative effect in therapeutic doses which was the common side effect of classical anti-histaminics.

Table-1: Behavioural Effects of Alcohlic Extract of Indula racemosa (60 mg/kg) in Mice

S. No.	Parameters	Control Mean±S.E. N=6	30 Minutes Percent Change Mean±S.E. N=6	60 Minutes Percent Change Mean±S.E. N=6	90 Minutes Percent Change Mean±S.E. N=6	120 Minutes Percent Change Mean±S.E. N=6
1	Horizontal activity	105.86±7.2	-31.99±7.2[a]	-16.51±2.3 [a]	-3.99±1.92 [b]	4.02±1.37 [b]
2	Total Distance	91.29±8.2	-29.32±2.6 [a]	-21.9±3.14 [a]	-8.37±2.22 [b]	-1.18±0.76
3	Movement Time	85.71±8.22	-28.12±4.22 [a]	-14.29±2.99 [a]	-3.76±0.33 [b]	-1.28±0.38
4	Stereotype Counts	61.44±5.22	-38.8±3.22 [a]	-30.17±2.76 [a]	-24.46±3.8 [a]	-7.28±0.38[b]
5	Marginal Time	57.01±3.99	-29.26±3.2 [a]	-28.48±2.6 [a]	-19.17±3.2 [a]	-17.5±3.66 [a]

[a]p<0.001, [b]p<0.05 (Paired t-test)

Table-2: Behavioural Effects of Alcohlic Extract of Indula racemosa (120 mg/kg) in Mice

S. No.	Parameters	Control Mean±S.E. N=6	30 Minutes Percent Change Mean±S.E. N=6	60 Minutes Percent Change Mean±S.E. N=6	90 Minutes Percent Change Mean±S.E. N=6	120 Minutes Percent Change Mean±S.E. N=6
1	Horizontal activity	105.86±7.2	-36.84±6.16[a]	-25.62±3.52 [a]	-19.55±8.22 [a]	3.36±1.31
2	Total Distance	91.29±10.86	-26.12±2.36 [a]	-16.32±3.11 [a]	-9.32±2.26 [a]	-7.86±1.22 [a]
3	Movement Time	85.71±12.22	-32.44±2.68 [a]	-19.61±3.77 [a]	-10.26±2.11 [a]	-8.86±2.01 [a]
4	Stereotype Counts	61.44±6.54	-38.8±4.22 [a]	-31.26±3.68 [a]	-22.26±4.26 [a]	-11.26±2.11 [a]
5	Marginal Time	114.86±9.38	-29.99±3.11 [a]	-26.21±9.66 [a]	-17.61±3.11 [a]	-9.76±1.71 [a]

[a]p<0.001 (Paired t-test)

Table-3: Behavioural Effects of Alcohlic Extract of Indula racemosa (240 mg/kg) in Mice

S. No.	Parameters	Control Mean±S.E. N=6	30 Minutes Percent Change Mean±S.E. N=6	60 Minutes Percent Change Mean±S.E. N=6	90 Minutes Percent Change Mean±S.E. N=6	120 Minutes Percent Change Mean±S.E. N=6
1	Horizontal activity	84.96±3.66	-55.59±5.99[a]	-36.62±7.22[a]	-13.25±3.7[a]	-10.63±2.26[a]
2	Total Distance	75.81±7.89	-42.55±4.55[a]	-34.36±6.21[a]	-19.61±2.66[a]	-11.26±1.78[a]
3	Movement Time	57.73±6.52	-37.5±2.22[a]	-31.00±6.33[a]	-21.63±3.44[a]	-12.96±3.22[a]
4	Stereotype Counts	201.81±7.22	-32.5±1.99[a]	-27.26±4.17[a]	-19.21±3.76[a]	-11.26±1.78[a]
5	Marginal Time	89.49±2.76	-30.26±3.82[a]	-25.19±7.60[a]	-20.21±2.17[a]	-10.26±1.76[a]

[a]p<0.001 (Paired t-test)

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