
Acute - Toxicity, Anti-Inflammatory and Bronchial Smooth Muscles Investigation of *Sisymbrium Irio* Linn (Seeds) in Experimental Animal Models

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Abstract: *Sisymbriumirio* Linn (Seeds) are used in folk medicine for a treatment of inflammation, arthritis, expectorant, stimulant and asthma.,externally used as stimulating poultice.Acute toxicity study of *Sisymbriumirio* Linn seeds was performed on mice (Swiss) and albino rats (Wistar strain),given single doses 10,100,200,500 and 1000mg/kg,po. showed normal behavior and no mortality up to 14 days. *Sisymbriumirio* Linn seeds (100-200x7days or 100-200x3days or 50-200mg/kg,po single dose),pretreatment time , 60 min showed significant sub-acute anti-inflammatoryprotection against Cotton pellet induced granuloma formation, no analgesic activity,decreased swim stress immobility in mice indicating some degree of antidepressant activity.*Sisymbriumirio* Linn seeds protected guinea-pigs against bronchospasm induced by histamine aerosol. The steroids, glycosides , alkaloids, and flavonoids present in the plant appear to be of chemotherapeutic interest.

Keywords: *Sisymbriumirio* : Acute- toxicity : Anti-inflammatory : Bronchospasm : Swim –stress immobility

1. INTRODUCTION

*Sisymbriumirio*Linn (Family:Cruciferae) is commonly known as*Khakshi*, which is found in various parts of India .*Sisymbrium*species is world- wide distributed from U.K., Europe,. Mediterranean Islands, North America, Caucasia, Middle East to entire Western Pakistan. In relation to India it grows in the cities of Srinagar and Jammu, Punjab (Siwalik range),Northern part of Rajasthan, Delhi area and in the Western part of U.P. uptoRanikhet and Lucknow[1].*Sisymbriumirio*Linn seeds have employed as a folk medicine remedy for inflammation and rheumatoid[2] antipyretic, analgesic and anti-microbial activities [3].*Sisymbriumofficinale* has a role in treatment of voice disorders[4].Ethanol extracts of *S. irio*seeds reported phytotoxic, cytotoxic and insecticidal activities [5].*Sisymbriumthellungii* showed antioxidant activity[6] Three flavonoids and two sitosterols were isolated from seeds and aerial parts of *Sisymbriumirio* L[7].This genus was found to contain flavonoids [8,9,10], alkaloids, anthraquinones[11], oils, steroids [12] and glycosides [13].Saudi Arabia species of *Sisymbriumirio* Linn from aerial parts ,isolated ten flavonoids with anti-oxidant properties[14].

The present investigation was conducted to study in detail *Sisymbriumirio* Linn (Seeds) in view of its medicinal importance in folklore medicine.

2. MATERIALS AND METHODS

2.1 Animal and Drug Administration

After approval of Institutional Animal Ethical Committee (IAEC), the present study was conducted in the Department of Pharmacology,NRIADD, Kolkata on inbred Albino mice (Swiss) 15-20g ,Albino rats (Wistar Strain)100-200g and Guinea-pig 300-450g.They were kept in the departmental animal house in individual cages at an ambient temperature of $26 \pm 3^{\circ}\text{C}$ and 60- 70% relative humidity with 12h:12h light:dark cycles.They had free access to standard rodent pellet diet (NIN, Hyderabad) and drinking water (Kinley) during the entire study period. The food was withdrawn 18h prior to surgical procedure, however, water was allowed *ad libitum*.

2.2.1 Plant Material

The *Sisymbrium irio* Linn (Seeds) was procured from the local market and identified in the Department of Pharmacognosy, NRIADD, Kolkata, peripheral Institute of Central Council for Research in Ayurvedic Sciences, Govt. of India. A few mg of powdered drug was warmed with Chloral hydrate, washed and mounted in glycerine. A few mg of powder was cleared in 4% KOH, washed and mounted in glycerine. A few mg of powder was washed in plain water, a drop of KI – solution was added and mounted. Camera Lucida drawings were done for the salient features of the drug. The voucher specimens have been preserved.

2.2.2 Extraction

Dried powdered (500g) *Sisymbrium irio* seeds were extracted by ethanol, and concentrated in a steam bath to a final yield of 90.0g (18.0% w/w). Chemical tests showed the presence of glycosides, steroids, alkaloids and flavonoids.

2.3.1 Acute-Toxicity Studies on Mice

Albino mice (Swiss: 3M+3F=6) weighing 15-20g were given graded doses of *Sisymbrium irio* Linn (Seeds) at the dose level of 10, 100, 200, 500, 1000 and 2000mg/kg, po and with Control. These animals were fasted 18 h prior to the experimentation. Both the test and control groups were received in a same volume of drug or vehicle control as per body weight. Experiments were conducted as per OECD guidelines-423 (Acute-Oral Toxicity-Single Dose) [15]. The animals were kept in observation for 96h upto 14 days for any gross behavioral changes and mortality. The animals were observed for symptoms ie writhing pilo-erection, salivation fur, lacrimation, convulsion, hyperreactivity etc continuously for the first 4h after dosing. The number of survival were noted after 24h. These animals were then maintained and observed daily for 14 days for further any toxicity. Complete postmortem was done on all survivors or if any animal found dead or moribund condition during the study period. Histopathological examination was performed on all collected tissues of individual animals.

2.3.2 Acute-Toxicity Studies on Rats

Albino rats (Wistar strain: 2M+3F=5) weighing 100-150g were given graded doses of *Sisymbrium irio* Linn (Seeds) at the dose level of 10, 100, 200, 500, 1000 and 2000mg/kg, po and with Control. These animals were fasted 18 h prior to the experimentation. Experiments were conducted as per OECD guidelines-423 (Acute-Oral Toxicity-Single Dose) [15]. The animals were kept in observation for 96h upto 14 days for any gross behavioral changes and mortality.

2.4. Anti –Inflammatory Activity (Cotton Pellet Granuloma Pouch)

Albino rats (Wistar Strain) weighing 150-200g were divided into 4 groups (N=6). Group I received double distilled water DDW (Control), Group II received Diclofenac sodium (13.5mg/kg, po) served as Standard Control and Group III and IV was given *Sisymbrium irio* at the dose level of 100 & 200mg/kg, po X 7 days. Cotton pellet was weighed 20mg sterilized and in a hot air oven at 120⁰C for 2h, then implanted bilaterally in region of rat under light ether anesthesia and stitched properly [16].

At the end of drug treatment cotton pellet were taken out by dissection, placed in petri dish and placed in 70⁰C oven for over- night and weight after cooling. Increase in the dry weight of the pellets was taken as measure of granuloma formation and compared with Control and Standard Control. Cotton pellet induced granuloma the average weight of the pellets of the Control group and Standard control as well as of the test group was calculated. The percent change of granuloma weight relative to control group was determined.

2.5 CNS Activity

2.5.1 Analgesic Activity (Writhing-Test)

The male mice (N=6 in each group) were pretreated with *S. irio* seeds at the dose level (50, 100 & 200mg/kg, po) and Control group received (DDW) 60 min prior to the experiments. The writhing induced by freshly prepared 0.6% Acetic acid (ip) in mice within 3-10 min. The number of writhing of the abdominal musculature and extension of the hind limbs were recorded for 10 min [17].

2.5.2. Swim Stress Immobility in Mice

The mice (N=5 in each group) pretreated with *S. irio* Linn seeds at the dose level (50,100 and 200mg/kg,po) and Control group received (DDW) 60 min prior to the experiments. Mice were made to swim in a 7x8x24 inch Perspex cage filled with water at 30°C for 15 min [18]. The immobility phase of each mouse was recorded and compared with that of the control.

2.6. Aerosol-Induced Bronchospasm in Guinea-pig

The guinea-pig exposed to an aerosol of 1% histamine show progressive signs of difficulty in breathing leading to convulsion and death. The time until signs of convulsion appeared is called pre – convulsion time [19]. In the present experiments the criterion used was time to onset of dyspnoea and percent protection was calculated. *S. irio* Linn seeds was administered (100 & 200mg/kg,po) once a day for 3 days. Actual experiment was done on day 3, 1h after *S. irio* administration.

2.7 Statistical Analysis

All the data was analyzed by student's t-test followed by ANOVA.

3. RESULTS AND DISCUSSION

3.1 Acute-Toxicity Studies on Mice

All animals treated with different doses of *Sisymbrium irio* showed normal behavior and No mortality was recorded up to 14 days, except 2000mg/kg dose 33.3% mortality recorded. After postmortem ,histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.2 Acute-Toxicity Studies on Rats

All animals treated with different doses of *Sisymbrium irio* showed normal behavior and No mortality recorded up to 14 days , except 2000mg/kg dose 20.0% mortality recorded. After postmortem ,histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.3. Anti –Inflammatory Activity (Cotton Pellet Granuloma Pouch)

The results are summarized in Table 1. *Sisymbrium irio* Linn seed doses 100 and 200mg/kg significantly inhibited and compared with control and standard drug diclofenac sodium.

3.4 CNS Activity

3.4.1 Analgesic Activity (Writhing-Test)

The results are summarized in Table 2. All the doses 50, 100 and 200mg/kg showed insignificant writhing against 0.6% Acetic acid (ip) (No analgesic activity).

3.4.2 Swim-Stress Immobility in Mice

The results are summarized in Table 3. All the doses 50, 100 and 200mg/kg showed a tendency to decrease immobility significantly.

3.5 Aerosol-Induced Bronchospasm in Guinea-pig

The results are summarized in Table 4. Both the doses (100 and 200mg/kg,po X 3days) protected guinea –pigs against bronchospasm induced by histamine aerosol when administered 1h prior the aerosol challenge.

Sisymbrium irio Linn seeds ,apart from divers uses in folk medicine, has recently been shown to possess anti- inflammatory, analgesic and antioxidant properties. The acute toxicity studies indicate that *Sisymbrium irio* Linn seeds have a significant margin of safety in mice and rats. The present study on sub- acute anti- inflammatory activity induced by cotton pellet granuloma pouch showed significant inhibition and compare with diclofenac sodium. The Porsolt swim stress immobility model is widely used to screen anti- depressant activity .The present finding indicate that decreasing swim stress immobility may reflect the presence of antidepressant activity [18], further investigation is required to reach a definite conclusion. The data of the present study indicate that *S. irio* protects guinea -pigs against histamine induced bronchospasm. Bronchial hyperreactivity is a characteristic

feature of bronchial asthma and immune responses as well as inflammatory reaction play an important role in causation of bronchial hyperreactivity .Therefore ,the finding suggests a protective role of *S. irioin* bronchial asthma.This finding is also in accordance with the earlier reports of anti-inflammatory[2],immunomodulatory and adaptogenic activities[20] in the light of the role of immune response and inflammatory processes in bronchial asthma.

Table 1. Anti-inflammatory effects of *Sisymbriumirio*Linn seeds by Cotton Pellet- induced Granuloma pouch Weight. Values are mean± SE Weight (mg) . Figures in parentheses indicate number of animals used.

Treatment (mg/kg ,po)	N	Weight of dry Cotton Pellet Granuloma	% inhibition
Control (DDW)	6	29.47 ± 0.028	-
DICLOFENAC SODIUM 13.5	6	12.32 ± 0.05 ^c	58.19 ^b
<i>Sisymbriumirio</i> 100	6	20.16± 0.007 ^a	31.59 ^b
200	6	9.62 ± 0.01 ^a	67.35 ^b

^ap<0.001 in respect to control.

^bp< 0.001 in respect to Standard Control

Table2. Analgesic effects of *Sisymbriumirio* Linn seeds by Writhing test induced by 0.6% Acetic acid in albino mice. Values are mean ± SE% writhing. Figures in parentheses indicate number of animals used.

Treatment mg/kg,po	N	% Writhing
Control (DDW)	6	30.18± 1.22
<i>Sisymbriumirio</i> 50	6	33.56± 5.71 ^{NS}
100	6	35.25± 8.69 ^{NS}
200	6	40.76±9.32 ^{NS}

NS= Not significant in respect to control.

Table3. Effect of *Sisymbriumirio* Linn seeds on Swim stress (900 s duration) in albino mice. Values are mean ± SE immobile phase (s). Figures in parentheses indicate number of animals used.

Treatment mg/kg,po	N	Swim stress immobile phase (s)
Control (DDW)	5	367.24± 34.27
<i>Sisymbriumirio</i> 50	5	230.33± 28.19
100	5	165.15± 21.08 ^a
200	5	149.21± 22.13 ^a

^ap<0.001 in respect to control.

Table 4. Effect of *Sisymbriumirio* Linn seeds on 1% histamine aerosol induced- bronchospasm in guinea –pigs. Values are mean ± SE % delay. Figures in parentheses indicate number of animals used.

Treatment mg/kg,po x 3days	N	% delay in onset of dyspnoea
Control (DDW)	6	17.08± 1.66
<i>Sisymbriumirio</i> 100	6	62.05± 7.04 ^a
200	6	66.09± 1.92 ^a

^ap<0.001 in respect to control.

4. CONCLUSION

Acute toxicity of *Sisymbriumirio* Linn seed has safe upto the doses of 1000mg/kg and caused no mortality and normal behavior. The results of the present study reveal that significant anti-inflammatory activity, swim stress immobility and bronchoprotective role. *Sisymbriumirio* Linn seed is rich in glycoside, alkaloids and flavanoids. Pure isolates of active principles need testing toward identifying immunomodulatory drug therapy for bronchial asthma.

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