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STUDIES ON ETHANOLIC EXTRACT OF *SISYMBRIUM IRIO* LINN (SEEDS) ON *IN VITRO* RAT MAST CELLS

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ABSTRACT

SisymbriumirioLinn (Family: Cruciferae (Seeds) is folklore medicine commonly known as Khakshi , is known to have wide range of Pharmacological activity. The seed is used as expectorant, stimulant, asthma, externally used as stimulating poultice. We reported previously the protective action of Sisymbriumirioin experimental bronchial asthma. The present study was designed to assess the protective effect of Sisymbrium Irio seeds on in vitro mast cell stabilization and active anaphylaxis model in experimental animals. The present investigation of ethanolic extract of the seeds of Sisymbrium Irio was found to have protective actions against histamine aerosol induced on broncho- constriction, mast cell degranulation induced by Comp.48/80 and active anaphylaxis. The present finding of Sisymbrium Irio because seed is rich in glycosides, steroids, alkaloids and flavonoids components present in extracts. The steroids, flavonoids and alkaloids have been reported to be effective in clinical asthma.

INTRODUCTION

Sisymbrium irio 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. North America, 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. upto Ranikhet and Lucknow (Khoshoo, 1966). *Sisymbrium Irio* Linn seeds have employed as a folk medicine remedy for inflammation and rheumatoid (Bolus, 1983) antipyretic, analgesic and anti-microbial activities (Vohora *et al*., 1980). *Sisymbriumofficinale* has a role in treatment of voice disorders (Meyer *et al*., 1982). Ethanolic extracts of *S. irio* seeds reported phytotoxic, cytotoxic and insecticidal activities (Sumaira Shah *et al*., 2013). *Sisymbrium thellungii* showed antioxidant activity (Lindsey *et al*., 2002) Ethanolic extracts of *S. irio*seeds showed anti-inflammatory activity, swim stress immobility and bronchoprotective role (Singh, 2015; Singh, 2016).

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Three flavonoids and two sitosterols were isolated from seeds and aerial parts of *Sisymbrium irio* L (Khan *et al*., 1991).This genus was found to contain flavonoids (Itziar *et al*., 1982; Rizk *et al*., 1986; Lockwood and Fsharypuor, 1986), alkaloids, anthraquinones (Arayno and Zafor, 1983), oils, steroids (Soulier, 1994) and glycosides (Krets *et al*., 1987). Saudi Arebiaspecies of *Sisymbrium irio* Linn from aerial parts, isolated ten flavonoids with anti-oxidant properties (Al-Jaber, 2011). The present investigation was conducted to study in *Sisymbrium irio* Linn (Seeds) on *in vitro* mast cell stabilization and active anaphylaxis model in experimental animals.

MATERIALS AND METHODS

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 ± 3^0C and 60- 70% relative humidity with 12h:12h light: dark cycles. They had fee 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.*

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.

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.

Effect of *S. irio* on *in-vitro* mesenteric mast cell of rats

Albino rats (CF strain) were sacrificed by cervical dislocation. The abdomen was opened and mesentery of the jejunam and ileam were carefully exposed. The mesentery along with small pieces of jejunam or ileam were removed and placed in a petri dish containing oxygenated Ringer Locke`s solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5 and glucose 1.0 g/L of double distilled water ph 7.4) at $37.0^o \pm 0.5^oC$.Tissue transferred to different doses of *S.irio* (0.5, 1.0 & 2.0 mg/ml) for 30 min and then challenged by Comp 48/80(2.5 µg/ml) for 10 min.

The tissue was then stained 0.1% Toludine blue in 4% Formaldelyde in saline for 15-20 min (Norton, 1954). The tissue was next transferred and kept in acetone (two changes) and then mounted on slides. Before mounting, excess pieces of fats were trimmed and the mesentery was stretched from the edges with the help of a needle. Each cell was considered either disrupted or not disrupted. The term disrupted was selected instead of fragmented because granules were found around many cells which did not appear to be in fragments. The sole criterion for calling a cell disrupted was the presence of granules outside the cell. Many cell did not show extrusion of granules but appear swollen at low concentration of Comp. 48/80. For each dose concentration 100 to 150 mast cell were examined and average percentage of disruption was calculated.

***Effect of S. irioon mast cell degranulation in* actively sensitized rats**

Rats were sensitized by injecting subcutaneously 0.5 ml of horse serum with 0.5 ml of triple antigen containing 20,000 million Bordetellapertussis organism (CRI, Kasuli, India) (Gupta and Tripathi, 1973). The sensitized rats were divided into 4 groups of 6 animals each. Rats of group I received double distilled water and served as control. Rats of group II, III and IV were administered *S.irio* (0.5, 1.0 and 2.0 mg/kg, p.o.) once a day for 14 days. On day 14 rats were sacrificed 1h after treatment and the intestinal mesentery was taken for the study of mast cell. In vitro mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained and examined microscopically.

Statistical Analysis

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

RESULTS AND DISCUSSION

Effect of *S. irio* on *in-vitro* mesenteric mast cell of rats

The results are summarized in Table 1.*S.irio*pretreatment with three doses reduced mast cell degranulation significantly.

Table 1. Effect of *S. irio*[10,50 and 100mg/ml] and Comp.48/80 [2.5 µg/ml) on rat mesenteric mast cell.Values are mean± SE% degranulation. Figures in parentheses indicate number of animals used

Treatment [mg/ml +2.5 µg/ml Comp. 48/80]	N	% degranulation	% inhibition
Control (DDW)	6	14.32 ± 0.12	-
Comp.48/80	6	89.21 ± 0. 19[c]	-
Sisymbriumirio 10+ Comp.48/80	6	31.52± 0.67[a]	53.87[b]
50+ Comp.48/80	6	28.73± 1.89[a]	57.01[b]
100+ Comp.48/80	6	20.64 ± 1.33[a]	66.07[b]

[a]p<0.001in respect to Control.
[b]p< 0.001 in respect to Standard Control.

Table 2 : Effect of mast cell degranulation in actively sensitized rats .[*Sisymbriumirio*10,50 and 100 mg/kg x 14 days] . Values are mean± SE% degranulation. Figures in parentheses indicate number of animals used

Treatment [mg/kg,po,45min]	N	% degranulation
Control (DDW)	6	90.06 ± 0.32
Sisymbriumirio 10	6	56.12± 0.17[a]
50	6	60.32± 0.64[a]
100	6	65.07 ± 0.43[a]

[a]p<0.01 in respect of Control

***Effect of S. irioon mast cell degranulation in* actively sensitized rats**

The results are summarized in Table 2. All the three doses show decrease in mast cell degranulation in sensitized and

horse serum challenged model. *Sisymbrium Irio* Linn seeds, apart from divers uses in folk medicine, has recently been shown to possess anti- inflammatory, anti-depresent and protective role in bronchial asthma (Singh, 2015). Inhibition of mediator release from rat mesenteric mast cells by direct pre-treatment with *S.irio*, in addition to earlier demonstrated protective systemic effect against histamine induced bronchospasm in Guinea –pigs (Singh, 2015)**.** The present findings of mast cell protective effects of *S.irio* in against Compound 48/80 challenge and horse serum challenge in sensitized rats are further elaborative of our earlier reported bronchoprotective action of *S.irio*. Therfore, the finding suggests a protective role of *S. irio* in bronchial asthma. This finding is also in accordance with the earlier reports of immunomodulatory and adaptogenic activities (Patrick, 2002) in the light of the role of immune response and inflammatory processes in bronchial asthma. The mast cell protective action of *S.irio* may well be attributed to its alkaloids, glycoside and flavonoids components.

Conclusion

The results of the present study reveal that *Sisymbrium Irio* Linn (Seeds) has significant broncho protective roleinmast cell degranulation induced by Comp.48/80 and active anaphylaxis. *Sisymbrium Irio* 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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