Ancient science of life Vol : XXIII (4) April, May, June – 2004 Pages 59 - 65

Spasmolytic activity of a herbal drug isolated from *Tephrosia purpurea* in guinea pigs

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Received : 16-8-2003 *Accepted : 12-2-2004*

ABSTRACT **:** We investigated the spasmolytic activity of herbal drugs isolated from Tephrosia purpurea on guinea pigs for the treatment of asthma in India. For this investigation, the herbal drug was extracted with 70% ethanol in soxhlet apparatus. After purification and isolution, the drug was used in experimental animals to observe prophylactic activity. For anaphylactic activity, horse serum 0.5 ml along with triple antigen (0.5 ml) was induced in guinea pigs. To observe prophylactic activity, male guinea pigs weighing about 250-450 gms were killed by cervical dislocation and the trachea was isolated. Each trachea was cut in to six segments. Each segment consists of three cartilage rings. Each end of tracheal muscles was attached to the bronchospasm transducers for isometric recording of the tension charges on a polygraph. The results of experiments clearly showed the spasmolytic activity of the drug. The preliminary phytochemical investigation, however shows the presence of glycoside saponins.

Key words : *Tephrosia purpurea*, spasmolytic, polygraph, Bronchospasm.

INTRODUCTION

Although the importance of natural products in modern pharmacology is decreasing, a substantial part of all drugs are still based on compounds originally isolated from nature. Many pharmacologically active natural products come from plants that have been used in traditional medicine. As the number of asthmatics has increased during the last decades (Barnes et. al., 1988), mainly due to air pollution and improper ventilation, the development of new drugs to treat asthma has become an important task for the pharmaceutical industry. A considerable number of plants (lewis & Elvin Lewis, 1977) are traditionally suggested to be effective against diseases of the respiratory system, either as antitussive agents or against bronchitis or asthma, even if the importance of traditional medicine in northern Europe is not comparable with other parts of the world.

The assay used to screen the extracts is a standard in vitro assay to measure the relaxing effect against carbacholine induced contraction of the guinea pig trachea in an organ bath, and it is routinely used in pharmacological laboratories to determine the bronchodilatory effect of drugs (waldeck et.al. 1986). The selection of plants was made according to the following criteria:

a. There was a report of traditional utilization of the plant to treat respiratory diseases (Lewis and Elvin Lewis, 1977; Hegnauer, 1962 - 1973; Duke, 1985; Geothberg et.al., 1982).

b. There was not any reference in the literature to investigation of the smooth muscle relaxing properties of the plant.

c. The plant must be readily available, either from natural habitats in the vicinity of our laboratary or from local cultivation.

The present paper reports the effect of alcoholic extract of *Tephrosia purpurea* of family Leguminosae against isolated tracheal muscles of guinea pigs.

MATERIAL METHODS

2.1 Plants material: *Tephrosia purpurea* of family leguminosae is an annual shurb widely distributed in india. The leaves of the plants were collected from the college campus during winter months (Fig 1). The powdered material of 40-60 mesh size was soxhlet with 70% ethanol which yielded 10-45%. The yellowish green crude extract was subjected under reduced pressure in vacuum evaporator, which gave dark green semisolid crude. Chromatographic seperation, yielded five fractions. Fraction II upon purification, isolation, acidhydrolysis, and methylation gave a powdered material. This material was sent to the CDRI Lucknow for spectral analysis. This analysis has been done. All spectral graphs were sent to RSIC Madras, for structural elucidation by analysing the spectrum. Tentative structure was determined which shows Glycoside Saponin(fig. 2)

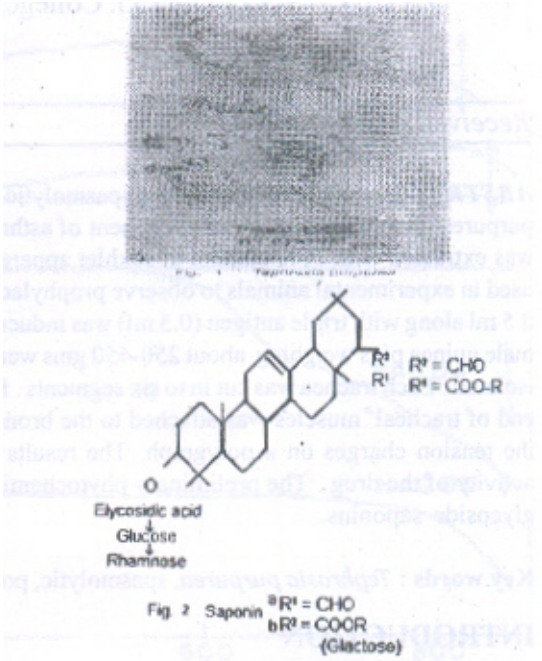

Fig. 2 Saponin aR¹ = CHO bR² = COOR

2.2 Animal material: For experimental work of asthma, guinea pigs were used. Guinea pigs were collected from Mahu City, Near Indore (m.p) India. The guinea pigs is shown in fig (3).

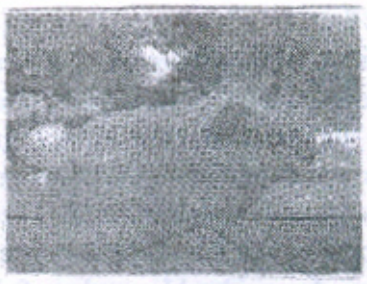
Fig 3 Guinea Pig

2.3 Anaphylactic activity : For the induction of asthma, forty eight guinea pigs were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml triple antigen containing 20, 000 million *Bordetella pertussis* organisms (Serum Institute of India ltd. Pune).The senstized

animals were divided into six groups of the eight animals each. Guinea pigs of group I received water vehicle and served as control guinea pig of group II,III, IV& V were administered herbal drug isolated from Tephrosia purpurea at 25,50,75 & 100 mg/ kg.p.o.. Respectively, once a day for 14 days . Group VI of guinea pigs received 0 mg/kg of prednisolone (reference drug) orally for the same duration. On day 14th, the guinea pigs were sacrificed two hours after treatment and the intestinal mesenteries was taken for the study on the mast cells. Mesenteries of the sacrificed guinea pigs along with intestinal pieces were kept in Ringer - Locke's solution at 37°c. The mesenteric pieces were challenged with 5% horse serum for 10 minute after which the mast cell were stained and examined microscopically for the number of intact and degranulated mast cells (Mitra et.al.,1999)

Fig. 4

Fig. 5

Fig. 6

2.4 Tracheal chain preparation (TCP)

Male guinea pigs of about 200-400 gm were used and kept under standard laboratory conditions in all experiments. The animals were stunned by a blow on the head and exsanguinated. The trachea was dissected out, freed from connective tissues and cut into the sections of two cartilage rings. (fig.3) Cotton threads were fastened to both ends of the cartilage bridge, which was then cut open ventrally. The preparation was maintained in an organ jacketed water bath (fig.4) 40 ml in a Ringer locke solution and allowed to stabilize for 1 hr at a basal tone of 5 ml. The solution was maintained at 37°C and oxygenated with mixture of 5%, Co_2, in O_2. The measurements of isometric contraction were made with the aid of a grass modal 7 D polygraph using FTO_3 force transducers.

RESULTS AND DISCUSSIONS

Tephrosia purpurea which is locally known as "Sarpunkha" causes relaxation in the isolated guinea pig trachea. Acetylcholine and histamine have large contractile effects on tracheal chains and contraction are dose dependent. The drug isolated from *Tephrosia purpurea* did not interact with the acetylcholine but it inhibited the contractile action of histamine on isolated tracheal chains. All the effects of 70% alcoholic extract of leaf of *Tephrosia purpurea* on isolated tracheal tissue are dose dependent and the action were prolonged with increase in doses. Water and alcoholic extracts of *Tephrosia purpurea* in 4 different doses that is 25, 50, 75 & 100mg/kg body weight/i.p. produces a dose dependent pre-convulsion time in each group. Each group served as its own control and blanked group was also used to assess to any variation in the response. The results have been given in table - 1, Fig (1)(a)&(b) and chymograph.

The effect of 70% alcoholic extract of leaf of Tephrosia purpurea on isolated tracheal

tissue are dose dependent and the action were prolonged with increase in doses Water and alcoholic extracts of Tephrosia purpose in 4 different doses, that is 25,50,75 and 100 mg/kg body weight, ip produces a dose dependent preconvulsion time in each group served its own control and balanced group was also used to assess any variation to the response. The results have been given in Table -I Fig (1)(a) & b and chymograph

A wide variety of pharmacologically active agents have been implicated in bronchial asthma. Histamine is one of the substances that produce instant bronchospasm - a characteristric feature of clinical pathophysiology of bronchial asthma. The bronchial muscles of guinea pig is extremely sensitive to histamine. (Rebuck,1974) and its respiratory anaphylaxis bears sufficient analogy with human and asthamatic syndrome. Experimental bronchospam provoked by inhalation of histamine has been widely investigated and evaluated to see the productive effect of antihistamine and other agents. Palit eta (1983) reported the antiasthamatic plant drugs from ancient Indian Ayurvedic Medicine and also noted dose depended antihistaminic effect of herbal drugs. In the recent year also Torres et.al. (2000) have noted the relaxant effect of a plant extract on vascular smooth muscles of the rat. Rojas et.al. (2000) have reported a biologically active substance in plant *Piper memthysticum* which inhibits airways and smooth muscles contraction.

Similarly Ko.et.al (2001) have reported that extract of *Petasites hybridus* have spasmolytic and antiasthmatic activity. In the present laboratory also Khare & Saxena (1999) have reported the leucocyte inhibiting activity in the methanolic extract of *Tephrosea purpurea.* The finding of present study therefore report the sapsmolytic does dependent activity in alcoholic extract of *Tephrosea purpurea* rather than water extract.

ACKNOWLEDGEMENT

Financial assistance received from the MAPCOST Project grant no.z - 15/93 is acknowledged with thanks.

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Table - 1

Effect of Herbal Drugs isolated from *Tephrosia purpurea* on mast cell degranulation in activity sensitized Guinea Pigs

Group	Treatment	Dose (Mg/Kg/P.O. for 14 days)	Percent inhibition of spasm release form mast cell	
			Alcoholic (70% Extract of Tephrosia purpurea	
			Exp.I (%)	Exp.II(%)
I	Control	-	26.01	26.01
II	Fraction of T.P	25	19.00	24.00
III	--- '''---	50	33.00	49.00
IV	--- '''---	75	44.00	53.00
V	--- '''---	100	65.00	68.00
VI	Prednisolone	10	76.27	76.27

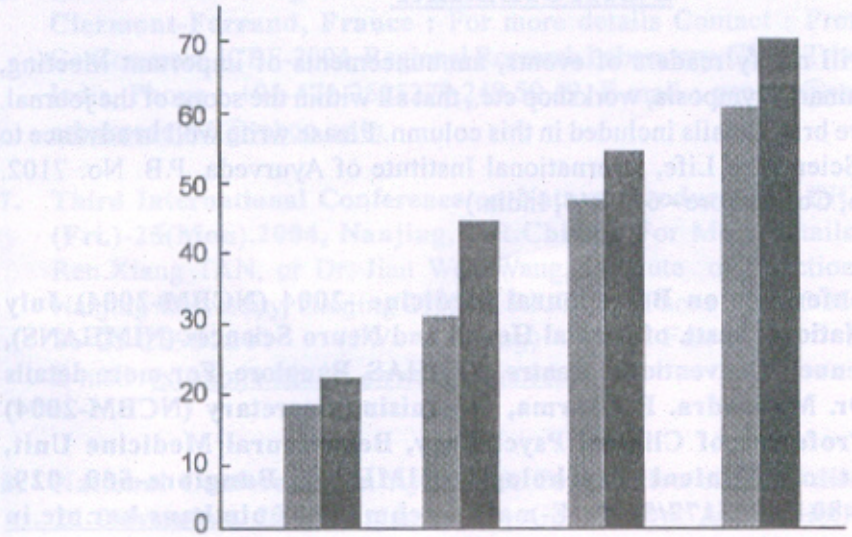

Fig Showing % inhibition of histamine release from mast cell in water extract

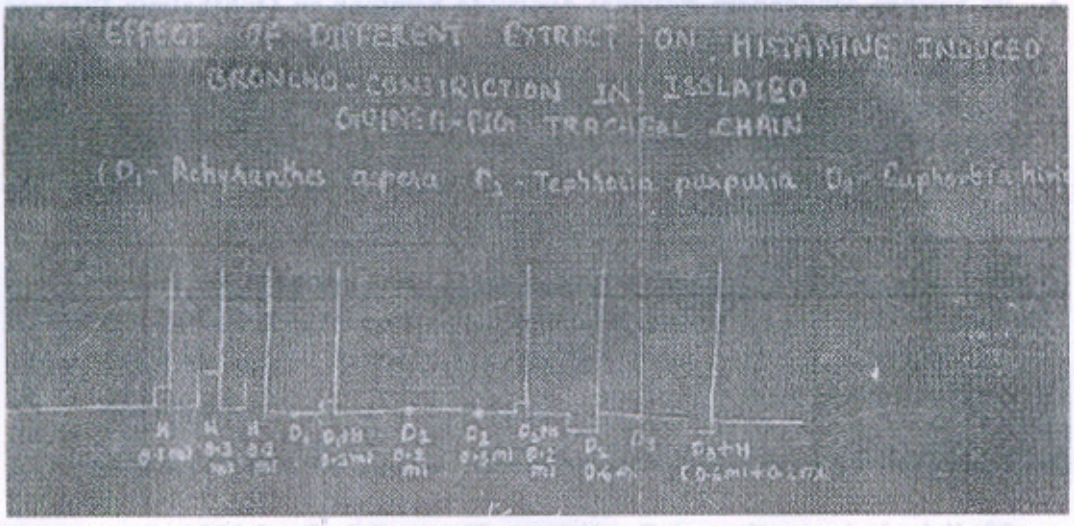

Fig. 7. showing Chymographic representation of *Tephrosia purpurea*