3. MATERIALS AND METHODS

3.1 - Plant material

Dried seeds of *Trigonella foenum- graecum* (fenugreek) and dried rhizome of *Zingiber officinale* (ginger) were purchased commercially in May 2004. Rhizomes of *Panax pseudoginseng* were collected from Singalila range of Darjeeling. They were authenticated by Dr. S.C Pal of Dept of Pharmacognosy, NDMVP Samaj's college of Pharmacy, Nasik and a voucher specimen was deposited at the Botonical Survey of India, Pune, for future reference. The *Korean ginseng* extract (KGE) was obtained as a gift sample from Glenmark Pharmaceuticals, Nashik. It was manufactured by Pangin Biotech Co. Ltd, Korea.

3.1.1 - Preparation of extract

One kg of *Trigonella foenum-graecum* seeds was crushed to a coarse powder and defatted with petroleum ether (60°- 80° C) using Soxhlet's extractor in two batches. The marc obtained was extracted with methanol. The methanol extract (ME) (5.85 % yield) was separated into acetone soluble (0.95 % yield) and acetone insoluble (4.90 % yield) fractions. The acetone soluble fraction was charged into chromatography column of neutral alumina. The column was eluted with ethylacetate and methanol to obtain ethylacetate fraction (EAF) (0.002 % yield) and methanolic fraction (MF) (0.06 % yield). The phytochemical tests indicated the presence of alkaloids, steroids, tannins and saponins (Khandelwal, 2003) (Table 9).

One kg of dried rhizome of *Zingiber officinale* were finely powdered and defatted with petroleum ether (60°- 80 ° C) by cold extraction process in two batches. The constituents of pet ether extract were separated by column chromatography (Evans, 2002). The pet ether extract (PE) and its toluene fraction (TF) were used for the investigation. The pet ether extract gave a yield of 3.38 % w/w, and toluene fraction gave a yield of 0.5 % w/w. The chemical tests indicated the presence of terpenes (Harborne, 1973; Khandelwal, 2003) (Table 9).

The standardized and controlled *Korean ginseng* slender tail roots was extracted 3 times under 70° for about 8 hrs in the extraction apparatus with 70% of ethanol. The extract was concentrated in vacuo at a reduced pressure of 500 mmHg- 600 mmHg under 60°-70° till the ginseng extract (KGE) was obtained. It contained 18% (W/W) of saponins. The phytochemical tests indicated the presence of glycosides, triterpenes and saponins (Khandelwal, 2003) (Table 9).

The rhizomes of *Panax pseudoginseng* subsp. *himalaicus* Var. augustifolius (1kg) were cut into small pieces and extracted with ethanol. The ethanol extract was concentrated to dryness in vacuo at a reduced pressure of 500 mmHg- 600 mmHg under 60°-70° to get a viscous residue (100gm). This was dissolved in ethanol and extracted successively with n-hexane and n-butanol. N-butanol extract (PPE) was found to contain glycosides, triterpenes and saponins (Khandelwal, 2003) (Table 9).

EAF of methanolic extract of fenugreek seeds, MF of methanolic extract of fenugreek seeds and TF of pet ether extract of ginger rhizome were subjected to HPTLC fingerprinting (Figure 10). TF was dissolved in chloroform and subjected to Gas chromatography mass spectroscopy (Perkin Elmer, USA) and the mass spectrum was obtained (Mendham, 2002). PPE was subjected to LC-MS analysis.

3.1.2 - Drugs and Chemicals

Serotonin hydrochloride (5-HT), meta-chlorophenylpiperazine (*m*-CPP; 5-$HT_{2A/2B/2C}$ agonist), Phenylbiguanide (PBG; 5-HT_3 agonist), Ketanserin (5-HT_{2A} antagonist), Noradrenaline (NA), Adrenaline (Adr), Phenylepherine (PhE), Acetylcholine (ACh), Urethane and Deoxycorticosterone acetate (DOCA-mineralocorticoid) were purchased from Sigma (Sigma Chemicals, St. Louis, USA). Fructose was purchased from SD Fine Chemicals. DOCA was dispersed in cottonseed oil. Diazepam (GABAa agonist) (Calmpose, Ranbaxy Laboratories, India), Ondansetron (5-HT_3 antagonist) (Emset, Cipla, India), Haloperidol (D_2 antagonist) (Searle, India), Lithium Sulphate (Glenmark, India) and Pentobarbital (GABA agonist) were used in the study. Pet ether (60°- 80° C), Ethyl acetate,

Methanol, n-butanol, and toluene were purchased from Modern Scientific, Nashik. The EAF of fenugreek seeds was suspended in PEG-400 (just sufficient to dissolve- not exceeding 0.005% of the total volume). Dilutions of ME and MF of fenugreek seeds, KGE and PPE were prepared in fresh distilled water. The pH of methanol extract and its ethyl acetate fraction was 6.4 and 7.5 respectively. The PE and TF of *Zingiber officinale* were suspended in Tween-80 (just sufficient to dissolve- not exceeding 0.05% of the total volume). Tween 80 was used as a solubilizing agent as PE and TF were insoluble in water. The pH of PE, TF, PPE and KGE were between 6 and 7. All drug solutions were freshly prepared in distilled water before each experiment. PBG was solubilised using Tween 80 (less than 0.5% of the total volume). 10% fructose solution was freshly prepared in distilled water. The extracts and the drugs were administered intraperitoneally in all acute experiments, and orally in all chronic experiments

3.1.3 - Animals

Male albino mice (22-25 gm) were housed into groups of five at an ambient temp of 25±1°C and Wistar rats (200-250 gm) of both sexes were housed into groups of two at an ambient temp of 25±1°C. Animals had free access to food (Hindustan Lever, India) and water. Animals were deprived of food but not water 4h before all acute experiments. All experiments were carried out during the light period (08:00-16:00 h). The Institutional Animal Ethical Committee approved the protocol of all the experimental studies.

3.2 - ANXIOLYTIC STUDIES (Acute)

3.2.1 – Experimental Protocol

3.2.1a – *Trigonella foenum graecum* (seeds)

(ME- Methanolic extract of fenugreek seeds; EAF- Ethylacetate fraction of methanolic extract; MF- Methanolic fraction of methanolic extract)

Group 1: Control: Mice received 0.1 ml of distilled water i.p.

Group2: Mice received diazepam (1 mg/kg) i.p.

Group 3: Mice received either ME (30,100 mg/kg) or EAF (30,100 mg/kg) i.p.

3.2.1b – *Zingiber officinale* (rhizome)

(PE-Pet ether extract of ginger rhizome; TF-Toluene fraction of PE)

Group 1: Control: Mice received 0.1 ml of distilled water (containing Tween 80 whose volume did not exceed 0.05% of the total volume) i.p

Group2: Mice received diazepam (1 mg/kg) i.p.

Group 3: Mice received either PE (10,30,100 mg/kg) or TF (10,30 mg/kg) i.p.

3.2.1c – *Panax Pseudoginseng* (rhizome)

(PPE - n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes)

Group 1: Control: Mice received 0.1 ml of distilled water i.p.

Group2: Mice received diazepam (1 mg/kg) i.p.

Group 3: Mice received PPE (3,10 and 30 mg/kg) i.p.

3.2.1d – *Korean ginseng* (roots)

(KGE - Ethanolic extract of *Korean ginseng* roots)

Group 1: Control: Mice received 0.1 ml of distilled water i.p.

Group2: Mice received diazepam (1 mg/kg) i.p.

Group 3: Mice received KGE (3,10 and 30 mg/kg) i.p.

The above extracts and fractions were subjected to the following acute models of anxiety based on exploratory behaviour.

3.2.2 - Elevated plus maze

The Elevated plus maze (EPM) consisted of two open arms (25 x 5 cm) crossed with two closed arms (25 x 5 x 20 cm). The arms were connected together with a central square of 5 x 5 cm. The apparatus was elevated to a height of 25 cm (Lister, 1987). Mice in groups of 5 were treated with vehicle; diazepam (1mg/kg) or ME (30,100 mg/kg) or EAF (30,100 mg/kg) or PE (10, 30 and 100 mg/kg) or TF (10 and 30 mg/kg) or PPE (3, 10 and 30 mg/kg) and KGE (3, 10 and 30 mg/kg) 30 min before placing individually in the center of plus maze. The time spent in open arms, entries in open and closed arms were recorded for a period of 5 min.

3.2.3 - Light/ Dark Apparatus test

Two equal sized boxes (20 x 20 x 14, one dark and the other lit) were connected with a tunnel (5 x 7 x 10 cm) (Belzung *et al.*, 1987). Mice in groups of 5 treated with vehicle; diazepam (1 mg/kg) or ME (30,100 mg/kg) or EAF (30,100 mg/kg) or PE (10, 30 and 100 mg/kg) or TF (10 and 30 mg/kg) or PPE (3, 10 and 30 mg/kg) and KGE (3, 10 and 30 mg/kg) 30 min before were placed individually in the lit area. The number of transitions in the light and dark box and the time spent in the lit box were noted for 5 min.

3.2.4 - Open field apparatus test

The apparatus consisted of wooden box (96 x 96 x 25cm). The floor of the box was divided into 16 equal squares (Turner, 1972a). Mice divided into groups of 5 each received vehicle; diazepam (1 mg/kg) or ME (30,100 mg/kg) or EAF (30,100 mg/kg) or PE (10, 30 and 100 mg/kg) or TF (10 and 30 mg/kg) or PPE (3, 10 and 30 mg/kg) and KGE (3, 10 and 30 mg/kg). After 30 min they were placed individually in one corner of the square. The number of rearings and the number of squares traversed were counted for 5 min.

3.2.5 - Hole board apparatus

The apparatus consisted of wooden box (40x40x25cm) with 16 holes (diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm (Clark *et al.*, 1971). Mice were treated with vehicle; diazepam (1mg/kg) or PPE (3, 10 and 30 mg/kg) and KGE (3, 10 and 30 mg/kg) 30 min before placing in the apparatus and the number of head pokes during 5 min were recorded.

3.3 – ANXIOLYTIC STUDIES (Chronic)

3.3.1 – Experimental Protocol

3.3.1a – *Trigonella foenum graecum* (seeds)

(ME- Methanolic extract of fenugreek seeds; EAF- Ethylacetate fraction of methanolic extract; MF- Methanolic fraction of methanolic extract)

Group 1: Control: Mice received 0.1 ml of distilled water p.o. for 15 days.

Group2: Mice received ketanserin (0.1 mg/kg) p.o. for 15 days.

Group 3: Mice received either ME (30,100 mg/kg) or EAF (10, 30 mg/kg) or MF (10, 30 mg/kg) p.o for 15 days

3.3.1b – *Zingiber officinale* (rhizome)

(PE-Pet ether extract of ginger rhizome; TF-Toluene fraction of PE)

Group 1: Control: Mice received 0.1 ml of distilled water (containing Tween 80 whose volume did not exceed 0.05% of the total volume) p.o. for 15 days.

Group2: Mice received ondansetron (1 mg/kg) p.o. for 15 days.

Group 3: Mice received either PE (10, 30, 100 mg/kg) or TF (10, 30 mg/kg) p.o. for 15 days.

3.3.1c – *Panax Pseudoginseng* (rhizome)

(PPE - n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes)

Group 1: Control: Mice received 0.1 ml of distilled water p.o. for 15 days.

Group2: Mice received diazepam (1 mg/kg) p.o. for 15 days.

Group 3: Mice received PPE (10, 30 and100 mg/kg) p.o. for 15 days.

3.3.1d – *Korean ginseng* (roots)

(KGE - Ethanolic extract of *Korean ginseng* roots)

Group 1: Control: Mice received 0.1 ml of distilled water p.o. for 15 days.

Group2: Mice received diazepam (1 mg/kg) p.o. for 15 days.

Group 3: Mice received KGE (10, 30 and 100 mg/kg) p.o. for 15 days.

The above extracts and fractions were subjected to the following chronic models of anxiety based on exploratory behaviour.

3.3.2 - Elevated T- maze

The apparatus is elevated 38.5 cm above the floor, has three arms of equal dimensions (30 x 5 cm). One arm was enclosed by walls (15 cm) and stood perpendicular to two open arms of the Elevated T maze (ETM) (Carvalho-Netto, 2004). The apparatus was cleaned with 70% alcohol after each trial. Mice in groups of five were administered orally ME (30 &100 mg/kg), EAF (10 &30 mg/kg), MF (10&30 mg/kg), PE (10, 30 &100 mg/kg), TF (10 & 30 mg/kg), PPE (10, 30 &100 mg/kg), KGE (10, 30 &100 mg/kg), Ondansetron (1mg/kg), Diazepam (1mg/kg), Ketanserin (0.1mg/kg) or vehicle for 15 days. In the pre-test session, on the 14^{th} day, individual mouse was pre-exposed for 30 min to one of the open arm. Pre-exposure shortens escape latency improving it as an escape index. On day 15, animals were individually placed at the distal end of the enclosed arm and the time taken to withdraw from this arm with all four paws was recorded (baseline). The procedure was then repeated for one additional trial (avoidance 1 and 2) using an inter-trial interval of 30 s. Thirty seconds after completion of the avoidance task, mice were individually placed at the distal end of the open arm, and time taken to withdraw from this arm was recorded (escape latency). The procedure was then repeated for one additional trial (escape 1 and 2) using an inter-trial interval of 30 s. A cut-off time of 300 s was employed for each trial (avoidance and escape test).

3.3.3 - Open field apparatus test

The apparatus consisted of wooden box (96 x 96 x 25 cm). The floor of the box was divided into 16 equal squares. Immediately after being tested in the elevated T-maze, each animal

was placed for 5 min in the open field apparatus for the evaluation of loco motor activity. During this time the total number of lines crossed was recorded (Turner, 1972a).

3.4 - OTHER TESTS

3.4.1- Experimental protocol

3.4.1a – *Trigonella foenum graecum* (seeds)

(ME- Methanolic extract of fenugreek seeds; EAF- Ethylacetate fraction of methanolic extract)

Group 1: Control: Mice received 0.1 ml of distilled water i.p. or diazepam (1 mg/kg) i.p or haloperidol (3 mg/kg) i.p. depending on the study.

Group 2: Mice received either ME (100 mg/kg) or EAF (30 mg/kg) i.p.

3.4.1b – *Zingiber officinale* (rhizome)

(PE-Pet ether extract of ginger rhizome; TF-Toluene fraction of PE)

Group 1: Control: Mice received 0.1 ml of distilled water (containing Tween 80 whose volume did not exceed 0.05% of the total volume) i.p or diazepam (1 mg/kg) i.p or haloperidol (3 mg/kg) i.p. depending on the study.

Group 2: Mice received either PE (50 mg/kg) or TF (30 mg/kg) i.p.

3.4.1c – *Panax Pseudoginseng* (rhizome)

(PPE - n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes)

Group 1: Control: Mice received 0.1 ml of distilled water i.p. or diazepam (1 mg/kg) i.p or haloperidol (3 mg/kg) i.p. depending on the study.

Group 2: Mice received PPE (50 mg/kg) i.p.

3.4.1d – *Korean ginseng* (roots)

(KGE - Ethanolic extract of *Korean ginseng* roots)

Group 1: Control: Mice received 0.1 ml of distilled water i.p. or diazepam (1 mg/kg) i.p or haloperidol (3 mg/kg) i.p. depending on the study.

Group 2: Mice received KGE (50 mg/kg) i.p.

The above extracts and fractions were subjected to the following tests.

3.4.2 -Behavioral assessment

To investigate the central actions of ME (100 mg/kg), EAF (30 mg/kg), PE (50 mg/kg), TF (30 mg/kg), PPE (50 mg/kg) and KGE (50 mg/kg) the method described by Irwin *et al* (1968) were employed. The procedure involved an initial phase of undisturbed observations and later a manipulative phase during which the animals were subjected to the least provoking stimuli. During the initial phase the animals were observed for body position, locomotion, rearing, respiration, tremors, gait, and in the later phase the effect on grip strength, passivity, pain response, righting reflex and lacrimation were observed. The animals were observed for 2 h after the treatment (Turner, 1972b).

3.4.3 -Neurotoxicity test

In this test, a knurled rod (2.5 cm in diameter) was rotated at a speed of 10 rpm. All animals were trained to remain on the rotating rod for 5 min. A normal mouse could maintain its equilibrium for long periods. In a drug treated mouse the neurological deficit was indicated by the inability of the mouse to maintain equilibrium for 3 min in each of the 3 trials as described earlier (Dunham and Miya, 1957). ME (100 mg/kg), EAF (30 mg/kg), PE (50 mg/kg), TF (30 mg/kg), PPE (50 mg/kg) and KGE (50 mg/kg) were tested for neurological deficit 30 min after the drug treatment. The control group received diazepam at a dose of 1mg/kg.

3.4.4 -Haloperidol induced catalepsy (DA mediated effect)

Rats were divided in groups of 5 each. The control group received haloperidol (3mg/kg). The other groups received ME (100 mg/kg), PE (50 mg/kg), PPE (50 mg/kg) and KGE (50 mg/kg) 30 minutes before haloperidol. The catalepsy was scored at 0, 30, 60, 90, and 120 min using the following scoring system: 0- rat moved normally when placed in table; 0.5- rat moved only when touched or pushed; 0.5- rat placed on table with front paws set alternately on a 3 cm high wooden block fails to correct the posture in 10 seconds for each paw (a total score of 1 per animal); 1.0- rat fails to correct posture in 10 seconds when front paws are placed on a 9 cm high wooden block, score 1 for each paw (a total score of 2 per animal). Thus for a single rat, a maximum possible cumulative score of catalepsy was 3.5. A lower score would mean apparently lesser degree of catatonia (Kulkarni *et al.*, 1980).

3.5. ANTIHYPERTENSIVE STUDIES (Acute)

3.5.1-Measurement of Blood pressure by invasive (Direct) method (Balaraman *et al.*, 1989)

Rats were anaesthetized with urethane (120mg/100gm, i.p) in groups of 5 each. Femoral vein was cannulated with a fine polythene catheter for administration of drugs. Blood pressure was recorded from left common carotid artery using pressure transducer by direct method on BIOPAC Data Acquisition System (BIOPAC MP30 System U.S.A) or Two channel UGO Basile blood pressure recorder. After 30 min of stabilization, the change in blood pressure was recorded to the following drugs:

i. Adr (1 μg/kg)
ii. NA (1 μg/kg)
iii. PhE (1μg/kg)
iv. 5-HT (1-3 μg/kg)
v. *m*-CPP (100μg/kg)

before and after

i. Methanolic extract of fenugreek seeds - ME (30mg/kg)
ii. Methanolic fraction of methanolic extract of fenugreek seeds - MF (10mg/kg)
iii. Pet ether extract of ginger rhizome -PE (10 mg/kg)
iv. Toluene fraction of PE of ginger rhizome -TF (3 mg/kg),
v. n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes –
vi. PPE (3 mg/kg)
vii. Ethanolic extract of *Korean ginseng* roots- KGE (3 mg/kg)
viii. Standard drugs: Ketanserin (10 μg/kg) and Ondansetron (1mg/kg) administration.

3.6. ANTIHYPERTENSIVE STUDIES (Chronic)

3.6.1 - Experimental Protocol

ME - Methanolic extract of fenugreek seeds
MF- Methanolic fraction of methanolic extract of fenugreek seeds
PE-Pet ether extract of ginger rhizome
TF-Toluene fraction of PE of ginger rhizome
PPE - n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes
KGE - Ethanolic extract of *Korean ginseng* roots

3.6.1.1 -DOCA-salt induced hypertension model:

Unilateral nephrectomized female animals (200-250 gm) were randomized into following groups of 5-6 animals each.

Group 1: Sham Control: Sham operated control rats received 0.2 ml. of cottonseed oil; s.c.; twice a week; for 4 weeks. Drinking water was replaced with 1% saline + 0.2 % KCl *ad libitum.*

Group 2: ME-30/ PE-50: Unilateral neprectomized rats received ME (30 mg/kg/day; p.o.) or PE (50 mg/kg/day, p.o.) and cottonseed oil (0.2 ml/rat/twice a week; s.c.) for 4 weeks. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 3: MF-15/ TF-10/ PPE-30/ KGE-30: Unilateral neprectomized rats received MF (15 mg/kg/day; p.o.) or TF (10 mg/kg/day, p.o.) or PPE (30 mg/kg/day, p.o.) or KGE (30 mg/kg/day, p.o) and cottonseed oil (0.2 ml/rat/twice a week; s.c.) for 4 weeks. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 4: DOCA: Unilateral nephrectomized rats received DOCA-salt, (15 mg/kg/twice a week; s.c.) dispersed in cottonseed oil, for 4 weeks. Drinking water was replaced with 1% saline + 0.2 % KCl *ad libitum.*

Group 5: DOCA+ME-30/ DOCA+PE-50: Unilateral nephrectomized rats received DOCA-salt (15mg/kg/twice a week; s.c.) dispersed in cottonseed oil and ME (30mg/kg/day, p.o.) or PE (50 mg/kg/day, p.o.) for 4 weeks. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

Group 6: DOCA+ MF-15/ DOCA+ TF-10/ DOCA+ PPE-30/ DOCA + KGE-30: Unilateral nephrectomized rats received DOCA-salt (15mg/kg/twice a week; s.c.) and MF (15 mg/kg/day; p.o.) or TF (10 mg/kg/day, p.o.) or PPE (30 mg/kg/day, p.o.) or KGE (30 mg/kg/day, p.o.) for 4 weeks. Drinking water was replaced with 1% saline + 0.2% KCl *ad libitum.*

3.6.1.2 -Fructose induced hypertension model:

Male wistar rats (200-250 g) were randomized and divided into following groups of 5-6 animals each.

Group 1: Control: Animals received no medication, but given distilled water for drinking.

Group 2: ME-100/ PE-50/ PPE-30/ KGE-30: Animals received ME (100 mg/kg/day, p.o.) or PE (50 mg/kg/day, p.o.) or PPE (30 mg/kg/day, p.o.) or KGE (30 mg/kg/day, p.o) and distilled water for drinking for 5- 6 weeks.

Group 3: F-10: Animals received 10% fructose solution instead of drinking water, *ad libitum* for 5-6 weeks.

Group 4: F-10 +ME-100/ F-10 +PE-50/ F-10 + PPE-30/ F-10 + KGE-30: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with ME (100 mg/kg/day, p.o.) or PE (50 mg/kg/day, p.o.) or PPE (30 mg/kg/day, p.o.) or KGE (30 mg/kg/day, p.o) for 5- 6 weeks.

3.6.2 -Induction of Hypertension

3.6.2.1 -Deoxycorticosterone acetate (DOCA) salt induced hypertension

Hypertension was induced experimentally in female Wistar rats (200-250 gm) by unilateral nephrectomy (Nagawa and Nasjletti, 1988). Rats were anaesthetized with ether. This was done by placing individual rat in a glass desiccator containing cotton balls immersed in diethylether. The moment the animal looses its righiting reflex it was removed. A lateral incision was made in the area overlying the kidney. The renal blood vessel was ligated with fine sterile silk thread and the kidney was removed. The incision was sutured and closed with Michel clips. All operated rats received an injection of ampicillin (10 mg/kg, i.p) daily for 5 days. Neosporin powder (Polymixin B sulfate BP, Zinc bacitracin BP, neomycin sulfate IP) was applied locally to prevent infection. One week later DOCA (15 mg/kg, twice a week; s.c; for 4 weeks) dispersed in cottonseed oil was injected to uninephrectomised rats. 1% saline + 0.2% KCl *ad libitum* was given instead of drinking water. In sham operated control animals, a similar procedure was performed except the treatment of DOCA.

3.6.2.2 -Fructose induced hypertension

Hypertension was induced experimentally in male Wistar rats (200-250 gm) by giving 10% fructose solution to drink *ad libitum* for five to six weeks. Fructose solution was prepared every two days by dissolving the fructose in distilled water. Ordinary tap water was given to control animals to drink throughout the whole experimental period (Vogel, 2002a).

3.6.3 - Measurement of blood pressure for DOCA and Fructose hypertensive models

3.6.3.1 -Measurement of Blood Pressure by noninvasive (indirect) method

For arterial blood pressure measurement using tail cuff method, rats were trained for at least one week until the blood pressure was steadily recorded with minimal stress and restrain. The first cardiovascular parameters were discarded and mean of five or six subsequent measurements were recorded. Cardiovascular parameters – systolic, diastolic, mean blood pressure and heart rate were measured weekly for five to six weeks by indirect non invasive tail cuff method using Letica 5002 Storage Pressure Meter (PANLAB BLOOD PRESSURE RECORDER model LE 2002 N, ITALY) (Vogel, 2002b).

3.6.3.2 - Measurement of Blood Pressure by invasive (direct) method

After completion of treatment schedule rats from each group were anesthetized with urethane (120mg/100g). Femoral vein was cannulated with fine polyethylene catheter for administration of drug. Tracheostomy was performed and blood pressure was recorded from left common carotid artery using pressure transducer by direct method on BIOPAC Data Acquisition System (BIOPAC MP30 SYSTEM, USA) (Vogel, 2002c) or UGO Basile Two-channel recorder. Heparinised saline (250 IU/mL) was filled in the transducer and in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, blood pressure and vascular reactivity to Noradrenaline (0.5μg/kg, 1 and 2 μM/kg), Adrenaline (0.5μg/kg, 1 and 2 μM/kg), Phenylepherine (0.5μg/kg), 5-HT (0.5μg/kg, 1 and 2 μM/kg), Phenylbiguanide (0.5 μg/kg) and ACh (0.5 μg/kg) were recorded depending on the study.

3.7. SOME STUDIES TO SUPPORT 5-HT HYPOTHESIS

ME - Methanolic extract of fenugreek seeds

EAF- Ethylacetate fraction of methanolic extract of fenugreek seeds

MF- Methanolic fraction of methanolic extract of fenugreek seeds

PE- Pet ether extract of ginger rhizome

TF- Toluene fraction of PE of ginger rhizome

PPE - n-butanol fraction of ethanol extract of *Panax pseudoginseng* rhizomes

KGE - Ethanolic extract of *Korean ginseng* roots

3.7.1. *Trigonella foenum- graecum*

Reversal of m-CPP induced anxiety

Mice in groups of 5 were treated with vehicle and different doses of EAF (30,60,120,240,480 mg/kg) and diazepam (0.5,1,2,4,8,16 mg/kg) after 30 min of *m*-CPP (1mg/kg) administration. After 30 mins they were individually placed in the centre of EPM and the time spent in open arms, entries in open and closed arms were recorded for 5 min.

Lithium induced head twitches (5-HT mediated effect)

Rats were treated with lithium sulphate (200 mg/kg i.p) 30 min after treatment with ME (100 mg/kg) or MF (30 mg/kg) or EAF (30 mg/kg). The number of head twitches was counted for 60 minutes after lithium sulfate treatment (Weilosz and Kleinrok, 1979).

3.7.2. *Zingiber officinale*

Gas chromatography mass spectroscopy

TF was dissolved in chloroform and subjected to Gas chromatography mass spectroscopy (Perkin Elmer, USA) and the mass spectrum was obtained (Mendham, 2002).

3.7.3. *Panax pseudoginseng and Korean ginseng*

Lithium induced head twitches (5-HT mediated effect)

Rats were treated with lithium sulphate (200 mg/kg, i.p) 30 min after treatment with PPE (30 and 100 mg/kg) and KGE (30 and 100 mg/kg). The number of head twitches was counted for 60 minutes after lithium sulfate treatment (Weilosz and Kleinrok, 1979).

Pentobarbital induced sleep (GABA mediated effect)

Mice were pretreated with PPE (30 mg/kg) or KGE (30 mg/kg) 30 min before pentobarbital (40 mg/kg, i.p). The sleeping time was measured as the period in which the mice lost the righting reflex after receiving pentobarbital (Turner, 1965).

Liquid chromatography mass spectroscopy

PPE was subjected to Liquid chromatography- mass spectrometry (Perkin Elmer, USA) and the mass spectrum was obtained (Luchtefeld *et al.*, 2004)

3.7.4 -*In- vitro* Studies:

After completion of treatment schedule in fructose induced hypertension model, rats from each group were sacrificed by stunning; fundus was removed and placed in Krebs solution. A strip of fundus was mounted in a bath containing Krebs solution. The physiologic salt solution had the following composition (mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and Glucose, 11. The physiologic salt solution had a pH of 7.4. It was warmed to 37 °C and aerated with 95% O_2 and 5% CO_2 (Carbogen). One end was tied to an aerator tube and the other end to a forced displacement transducer (Ugo Basile, Comerio Italy). Each strip was placed under optimum resting tension (1.5g) and allowed to equilibrate for 30 min with frequent changes of Krebs solution at 10 min interval. Contractile response to each dose of 5-HT was recorded for 90 sec for each tissue preparation on a Gemini Two Channel Recorder 7070 (Ugo Basile) (Goyal, 1999).

3.7.5 -Coagulation time

Blood was collected from retro orbital vein into a capillary (6-8 cm) from rats treated chronically with fenugreek, ginger and ginseng extracts at the end of antihypertensive study schedule (Fructose model). The capillary was the broken at into small pieces of 0.5 cm from its distal end at every 10 sec interval. Time of formation of fibrin thread of blood between broken pieces of capillary was noted (Goyal, 2002).

3.8 - Statistics

The mean ± SEM values were calculated for each group. One-way ANOVA/Kruskal Wallis test followed by Dunnets/ Tuckey's post-hoc/ Dunn's comparison test was used for statistical analysis. Values of $P<0.05$ were considered statistically significant.

Table 9

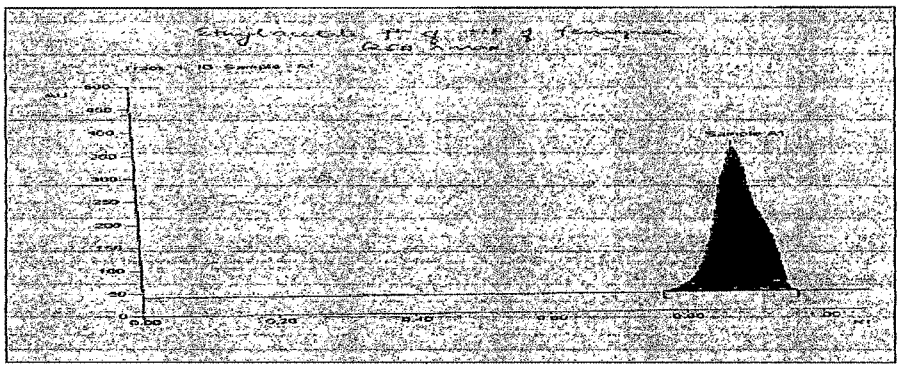
PHYTOCHEMICAL INVESTIGATIONS of the extracts (Khandelwal, 2003)

Test	Observation	Inference	ME	PE	PPE	KGE
Test for steroid *Liebermann Burchard Test* 2 ml of extract + 2 drops of acetic anhydride +1 ml of conc H_2SO_4	Green color at junction	Presence of steroids	+ve	----	----	----
Test for alkaloids 1 ml of extract + 0.2 ml of Mayer's reagent 1 ml of extract + 0.2 ml of Hager's reagent 1 ml of extract + 0.2 ml of Wagner's reagent	Reddish brown ppt Yellow ppt Reddish brown ppt	Presence of alkaloids	+ve	----	----	----
Test for glycoside 2ml of extract + 0.2 ml of β-napthol + few drops of H_2SO_4	Violet ring at junction	Presence of glycoside	----	----	+ve	+ve
Test for triterpenes 1 ml of extract + alcoholic $Fecl_3$ solution 1 ml of extract + vanillin sulfate spray reagent	Brownish color Violet color	Presence of triterpenes	---- ----	+ve +ve	+ve +ve	+ve +ve
Test for tannins 1ml of extract + 0.1 ml alcoholic $Fecl_3$ solution 1ml of extract + 0.1 ml of lead acetate solution	Blue-black color Reddish brown ppt	Presence of tannins	+ve	----	----	----
Test for saponins *Foam test* 1ml of extract solution + 10 ml of distilled water *Heamolytic test* 1ml of extract + 1 drop of blood on glass slide	Persistent foam observed Haemolytic zone appears	Presence of Saponins	+ve +ve	---- ----	+ve +ve	+ve +ve

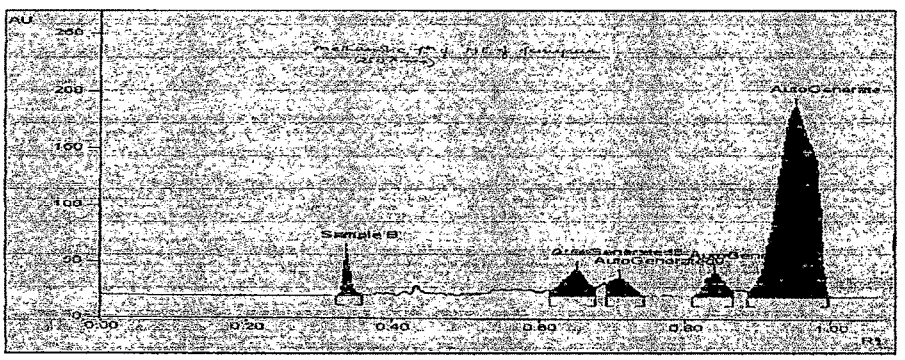
Figure: 10

HPTLC fingerprints of A) EAF of methanolic extract of fenugreek seeds. B) MF of methanolic extract of fenugreek seeds. C) TF of pet ether extract of ginger rhizome.

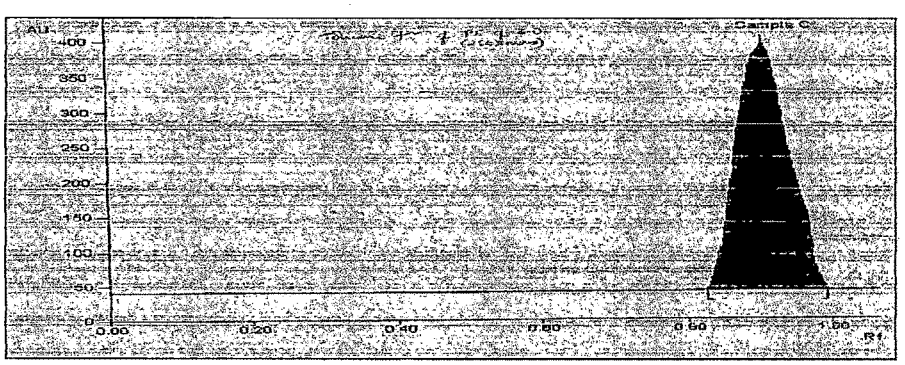
A)



B)



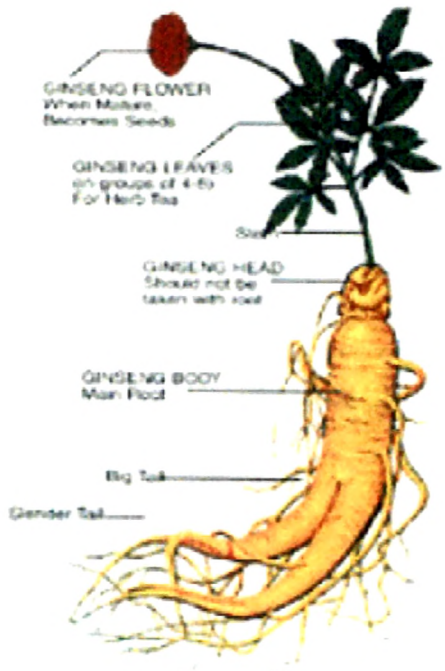
C)



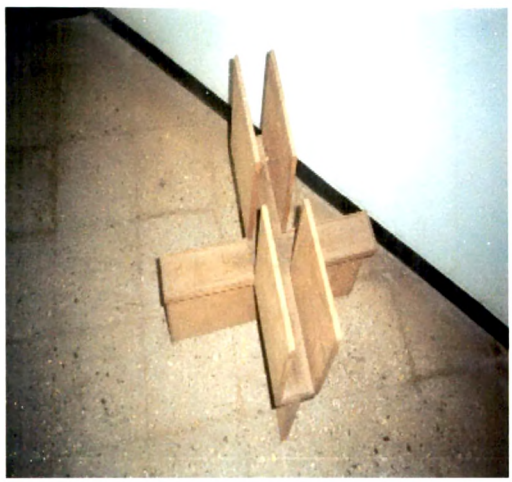
PHOTOGRAPHS

Fenugreek Seeds

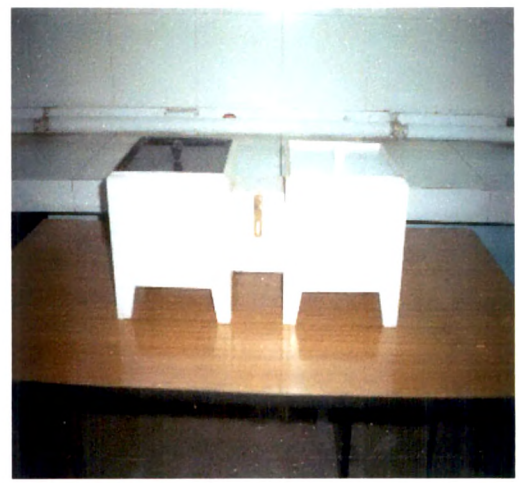
Ginger Rhizome



Ginseng Rhizome

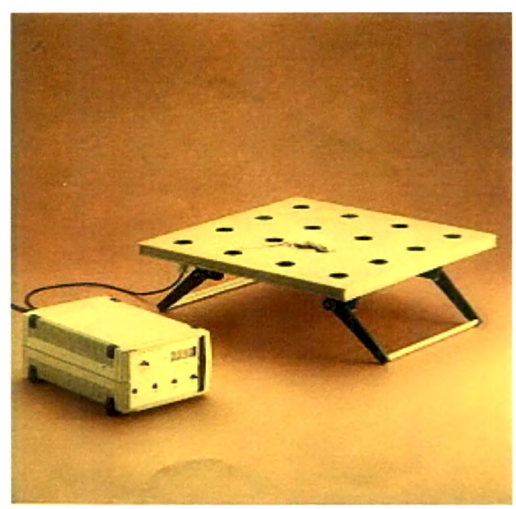


Elevated plus maze



Light/ Dark apparatus

Open field apparatus

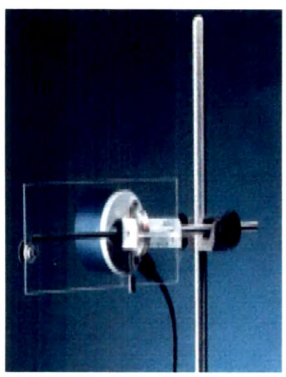


Hole Board apparatus

Two Channel Recorder Gemini

Blood Pressure transducer

Acquisition unit - MP 30



Isotonic Transducer

Rodent ventilator