5.DISCUSSION

Anxiety unlike other psychiatric conditions such as schizophrenia or depression is both a normal emotion and a psychiatric disorder. It commonly co-exists with other clinical conditions but can be diagnosed on the basis of symptoms and course of the disease. It is sometimes difficult to separate the normal from the pathological. However when the symptoms are disruptive and maladaptive enough to interfere with normal functioning of the individual, anxiety should be regarded as pathological one requiring requisite therapy. Another important cause is that of state-vs-trait anxiety. State anxiety is transient and is associated with identifiable stress, and remits once the stressor is eliminated. On the contrary trait anxiety is more persistent, the precipitating cause is often not apparent and the patient usually has a personality defect, which may have a inheritable component (Walker, 1990).

Traditionally, the availability of psychotropic drugs has fostored research in the field of psychopharmacology. The classical example is that of chlorpromazine; the clinical introduction of which initiated and stimulated research leading to the foundation of this discipline. Likewise, the advent of benzodiazepines led to intensive research and better scientific understanding of the basis of anxiety. Benzodiazepines introduced more than three decades ago, remain the anti-anxiety agents of choice because of their effectiveness and relative safety. However benzodiazepines are known to induce tolerance and physical dependance (Walker, 1990). This has led to the search of new and better anxiolytics. The recent introduction of anti-anxiety agents reducing central serotonergic activity, like the 5-HT_{1A} receptor agonist buspirone and the 5-HT_3 antagonist Ondansetron, have shifted the focus from the Benzodiazepine-GABA receptor complex to other neurotransmitter activity (Yocca, 1990). In the search for new effective and safer anxiolytics, pharmacologists have taken efforts to seek remedies for anxiety from plant kingdom.

In no other field of experimental pharmacology research is the issue more confounded than that of evaluating psychotropic agents. Whereas it is possible to devise experimental models of hypertension, diabetes mellitus, peptic ulcer, etc., it is obviously impossible to induce models of psychiatric illness in animals like rats and mice.

However some criteria have been suggested for validation of animal models of psychiatric disorders (McKinney, 1979). These are:

a. The including conditions should be similar (fear, conflict, etc)
b. The behavioral state should have some similarity with clinical anxiety.

c. The anxiety models should have similar underlying neurobiological mechanisms.

d. Clinically effective anti-anxiety agents should be effective in the model as well.

It is difficult, if not impossible, to maintain the first two criteria. For instance, exposing a rat to bright light might induce anxiety; whereas in man exposure to darkness may be more anxiety provoking. Likewise the classical symptoms of anxiety can be induced in primates but certainly not in rodents. The third criterion is also difficult to fulfill since the neurobiological mechanisms underlying human anxiety are still far from clear. Thus the fourth criterion is the one that most experimenters rely on, and can be regarded as pharmacological validation (Gray, 1987). A large number of methods have been employed to screen putative anti-anxiety agents using rats and mice as the experimental animal. We have however restricted the present work to methods, which can be employed in a reasonably equipped laboratory.

In the present study- (a) *Trigonella foenum graecum* (seeds) (b) *Zingiber officinale* (dried rhizome) (c) *Panax pseudoginseng* (rhizome) (d) *Korean ginseng* (roots) have been studied for their anxiolytic and antihypertensive actions. **The Indian variety of ginseng- *Panax pseudoginseng* has been compared with its Korean counterpart- *Korean ginseng* in all anxiolytic and antihypertensive studies.**

An extensive literature survey on the above plants suggested that:

a. *Trigonella foenum graecum* is well known for its appetite stimulant properties whose effect is mediated through 5-HT receptors. It also has antifatigue effects. Ghosal *et al.*, 1974 has worked on its antihypertensive effects without emphasizing on its 5-HT mechanism. Moreover, it is well known that a rise in endogenous 5-HT levels leads to anxiety and viceversa. This led to a hypothesis that anxiolytic and antihypertensive effects of this plant could possibly be mediated through its 5-HT mechanism.

b. *Zingiber officinale* is well known for its antiemetic effects (Yamahara *et al.*, 1989; Bone *et al.*, 1990) mediated through its 5-HT_3 antagonistic properties. This has prompted us to explore the antihypertensive effects of this plant through its 5-HT_3 antagonistic effects using Phenylbiguanide- a 5-HT_3 agonist, although literature survey has indicated that the aqueous ginger extract and its phenolic constituents lowers BP through a dual inhibitory effect

mediated via stimulation of muscarinic receptors and blockade of Ca^{++} channels (Ghayur *et al.*, 2005).

c. Ginseng, an adaptogenic is also being used as one of the commonly used over the counter herbal prescription in patients with cardiovascular disease (Pharand *et al.*, 2003). Korean red ginseng can improve the vascular endothelial dysfunction in patients with hypertension possibly through increasing the synthesis of nitric oxide (Sung *et al.*, 2000). Scanty work on *Panax pseudoginseng* has been reported with respect to its antihypertensive effects. Although these studies have reported different mechanism for their antihypertensive effects, no mechanistic studies involving the 5-HT have been hypothesised.

In search for better alternatives to antihypertensive drugs, the 5-HT_3 receptor antagonists, 5-HT_{1A} agonist and 5-HT_{2B} antagonists are currently being considered for their potential use in hypertension (Tsukomoto *et al.*, 2000; Shingala and Balaraman, 2005). Reports on anxiolytic studies on the above selected plants are very few (Vishwakarma *et al.*, 2002; Hwa-Young Cha *et al.*, 2004). I, therefore proposed to study anxiety and antihypertensive properties of the above plant species through its possible 5-HT mechanism.

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

The elevated plus- maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel, brightly– lit open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans (Rabbani *et al.*, 2003). It is also one of the most popular animal models for research on behavioral pharmacology of anxiety (Pellow *et al.*, 1985). In this test, rodents of most strains show a pattern of behavior characterized by open arm avoidance, a tendency that is generally suppressed by anxiolytics (Rodjers and Cole, 1994). Lister (1987) has validated the model in

mice and with different classes of substances and demonstrated that it is effective for examining anxiogenic like and anxiolytic effects of drugs. In the EPM test, decreased occupancy in the open arm and/or reduction in the open arm entries provide a measure of fear-induced inhibition of exploratory activity, which is attenuated by anxiolytics and increased by anxiogenic agents. (Pellow and File, 1986; Pellow *et al.*, 1987). Imaizumi *et al* (1994) have shown that diazepam (4 and 8 mg/kg) increased the open arm entries and time spent in open arm without changes in total arm entries. A putative anxiolytic 8-OH-DPAT decreased the total arm entries and the time spent on open arm at any doses tested (Masahiro, 1996). 5- HT_{1A} agonists are often called anxioselective anxiolytics and anxiolytic activity of buspirone has been clinically confirmed. Researchers have reported different effects of buspirone on EPM i.e anxiolytic (Dunn *et al.*, 1989; Soderpalm *et al.*, 1989; Kshama *et al.*, 1990; Lee and Rodgers, 1991; Luscombe *et al.*, 1992), non-effective (Wada and Fakuda, 1991), and anxiogenic (Pellow *et al.*, 1987; Moser, 1989; Redfern and Williams, 1989; Kostowski *et al.*, 1990; Klint, 1991; Critchley *et al.*, 1992). A drug may have both anxiolytic and anxiogenic activities and either of the activities may be dependent on experimental conditions including differences in the strain (Handley and Mc Blane, 1993a). Drugs must be carefully assessed on elevated plus maze test and therefore in the present study EPM is supported by other tests. In our study, we found that EAF of *Trigonella foenum- graecum* increased occupancy in open arm of the elevated plus maze and also exhibited diminished preference to the closed arm. It has been reported that *mCPP*, a 5-HT_2 receptor agonist is considered as a pharmacological tool for evaluating 5-$HT_{2A/2B/2C}$ receptor function. It was found to produce anxiogenic like effects in various animal and human studies (Curzon and Kennett, 1990; Germine *et al.*, 1992; Gibson *et al.*, 1994; Griebel, 1995; Griebel *et al.*, 1997; Kennett *et al.*, 1989; Klein *et al.*, 1991). It has been a model of anxiety in humans (Seibyl *et al.*, 1991; Shader and Greenblatt, 1995; Price *et al.*, 1995) and is known to produce anxiety like behaviours in rats and mice using a number of assays, including the elevated plus maze, t-maze and light dark box (Benjamin *et al.*, 1990; Bilkei-Gorzo *et al.*, 1998; Graeff *et al.*, 1998; Wallis and Lal, 1998). In the study using EPM different doses of EAF (30, 60,120,240,480 mg/kg) antagonized the effects of *m*-CPP (1mg/kg) in a dose dependent fashion. EAF increased occupancy in open arm of the elevated plus maze and also exhibited diminished preference for the closed arm. The antagonistic action of EAF against *m*CPP

substantites anxiolytic activity. An inverse U dose response [∩] relationship was observed. The antagonistic action of EAF against *m*-CPP substantiates anxiolytic activity. We also observed that animals treated with PE (50 mg/kg), TF (30 mg/kg), PPE (10 mg/kg) and KGE (10 mg/kg) significantly ($P<0.05$) spent more time in the open arm. PE (50 mg/kg), TF (30 mg/kg), PPE (10 mg/kg) and KGE (10 mg/kg) also increased the entries in both the open arms. **An inverse "U" dose–response [∩] was obtained which is the characteristic of anxiolytic agents** (Pal *et al.*, 1995; Hasenohrl *et al.*, 1998; Une, 2001). The results of PE and TF are in accordance with the earlier findings (Vishwakarma *et al.*, 2002).

The Light/Dark test is based on the natural preference of mice for a dark place rather than a brightly lit area. It is interesting that many studies in the Light/Dark test have been conducted using mice rather than rats. Crawley reported that rats were not respondent to treatment with diazepam in this paradigm (Crawley, 1985) and that their exploratory tendencies appeared considerably lower than in mice, suggesting that rats were not useful in this test. Mice have a conflict between their instinct for exploring novel environment and their natural aversion to lit areas. Anxiolytics increase their exploration in lit areas and anxiogenics decrease it (Masahiro, 1996). Explorative anxiety paradigms like these have been suggested to measure state anxiety (Belzung and Le Pepes, 1994). The light/dark test seems to be more sensitive to mechanisms implicating the 5-HT_3 receptor (Hascoet *et al.*, 2001). Anxiolytic benzodiazepines, chlordiazepoxide and clonazepam, increased light-to-dark transitions and total locomotion in mice at non-sedative doses but did not change the time spent. (Crawley, 1981; Crawley and Goodwin, 1980; Young and Johnson, 1991). In our study EAF (100 mg/kg) increased the time spent in lit zone but did not change the number of transitions. We also noted that PE (100 mg/kg) showed a significant ($P<0.05$) increase in the time spent in lit zone whereas PE (30 mg/kg), TF (30 mg/kg), PPE (10 mg/kg) and KGE (10 mg/kg) significantly increased the number of transitions.

In the open field test, EAF (100 mg/kg) showed an increased tendency to reach to the walls and rear rather than rearing without support. It also increased the squares traversed. PE (10 and 30 mg/kg) and TF (10 and 30 mg/kg) also significantly increased the number of squares traversed while; TF (30 mg/kg) significantly ($P< 0.05$) increased the rearings. Decrease in locomotion is indicative of diminished dopaminergic transmission, which may be secondary to the rise in 5-HT level caused by anxiogenic agents and vice-versa (Kahn *et al.*, 1988a;

Jones *et al.*, 1992). This observation is congruent with the data from EPM and LDA tests. The triols and diols of *Korean ginseng* have opposing action, generally stimulating and sedating respectively. It appears that in times of stress or fatigue the stimulating properties predominate. In these situations *Korean ginseng* will increase locomotor activity and the ability to respond to external stimuli (Wren, 1988; Mills, 1991; Teeguarden, 1994; Weiss, 1988; Bensky and Gamble, 1986; Hsou- Mou Chang and Pui- Hay, 1986; D'Angelo *et al.*, 1986). This is in accordance with our observation. In open field test, PPE (10 mg/kg) and KGE (10 mg/kg) showed a significant increase in squares traversed and increase in the tendency to reach to the walls and rear rather than rearing without support.

File and Wardil (1975) have assessed the anxiogenic and anxiolytic activity of some agents using the hole board test. We observed a significant ($P<0.05$) increase in head poking with PPE (3 and 10 mg/kg) and KGE (3 and10 mg/kg).

Enormous work has been carried out on 5-HT systems and anxiety models (Barrett and Vanover, 1993; Griebel *et al.*, 1997; Griebel, 1995; Handley and McBlane, 1993a). The vast literature indicates that conditioned procedures as well as more ethologically based tests are equal in revealing anxiolytic like effects of drugs targeting 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors (Griebel, 1995). On the other hand, anxiolytic like effect of 5-HT_3 receptor antagonists are in great part revealed by models based on spontaneous behaviours (Costall and Naylor, 1992; Griebel, 1996). Several 5-HT_3 antagonists have been studied clinically as potential therapeutics in the treatment of anxiety disorders (Oliver *et al.*, 2000; Roca *et al.*, 1995). As most of these tests were validated on their sensitivities to benzodiazepines and with the introduction of only one non-benzodiazepine agent (buspirone) into clinical practice, the validity of these models in testing for non-benzodiazepine potential anxiolytics has been queried. We have, therefore chosen diazepam as the standard anxiolytic drug. There is also a suggestion that each model reflects a different type of anxiety or fear and that the mechanism of 5-HT is different for each model (Griebel, 1996; Handley, 1995; Handley and McBlane, 1993b).

It has been indicated that an increase in serotonergic transmission can interfere with learning acquisition, and anxiety (Orgen, 1982). In our study we have observed ME (100 mg/kg) and MF (30 mg/kg) or EAF (30 mg/kg) diminished serotonergic transmission as observed from the decrease in the lithium induced head twitches (Weilosz and Kleinrok, 1979). A decrease

in brain 5-HT activity results in anxiolysis (Kahn, 1988a). It has been demonstrated that learning and memory storage can proceed normally despite depletion of brain DA (Davis, 1989). In our study facilitation of dopaminergic activity has been shown by all extracts till the completion of experiment.

In light of the above discussion, our findings suggest that alkaloid and saponin containing ethyl acetate fraction (EAF) and methanolic fraction (MF) of methanol extract of *Trigonella foenum-graecum* seeds possess anxiolytic activity probably by involving the 5-HT_2 receptors. Plants containing alkaloids have been reported to possess anti-anxiety effects (Kumar and Sharma, 2005; Elisabetsky and Costa, 2006; Cronin, 2003). Toluene fraction of pet ether extract of *Zingiber officinale* exerts its anxiolytic effect which is largely attributed to gingerol having 5-HT_3 antagonistic activity (Vishwakarma *et al.*, 2002) and saponin containing *Panax pseudoginseng* and *Korean ginseng* (Bhattacharya and Mitra, 1991, Hwa-Young Cha *et al.*, 2004) possess anxiolytic activity which may be contributed by its GABA mediated effect. A dose producing such an effect was not accompanied by it sedative or neurotoxic actions as observed with benzodiazepines.

The importance of demonstrating preclinical evidence of putative anti-anxiety activity cannot be minimized. However, it has to be recognized that, while benzodiazepines are effective in all the test parameters discussed, the newer 5-HT based antianxiety agents may have a different profile of action, being active in some paradigms and inactive in others (Oliver *et al.*, 2000). It has also to be appreciated that clinical anxiety includes generalized anxiety disorders, phobias and panic attacks and their treatment is not restricted to anxiolytics but include antidepressants as well. Different laboratories utilize varying sets of paradigms depending upon the expertise available. In our own set up with limitation of resources, we have found that a combination of simple tests like elevated plus maze, light/dark apparatus, open field apparatus, hole board apparatus, elevated T maze are robust enough to detect anxiogenic or anxiolytic actions of synthetic and natural products.

We also explored the potential of fenugreek seeds, ginger rhizome and ginseng roots in anxiety through the use of elevated T-maze and open field test. The ETM was derived from

elevated plus maze to investigate conditioned anxiety (Inhibitory Avoidance-IA) and unconditioned fear (Escape latency-EL) in the same animal; these responses have been related to generalised anxiety disorder (GAD) and Panic disorder (PD), respectively. The selective sensitivity of inhibitory avoidance and escape latencies to anxiolytic and panicolytic drugs, respectively, has encouraged the use of the ETM model for the study of the GAD and PD (Graeff *et al.*, 1993; Graeff *et al.*, 1998; Viana *et al.*, 1994). Previous studies (Graeff *et al.*, 1996; Viana *et al.*, 1994) revealed that the initial latency to leave the open arm was not significantly different from the first latency to withdraw from the closed arm. It is likely that exploration interferes with open arm escape. Therefore, animals were also subjected to open field test inorder to avoid confusing results due to treatment effects on loco motor activity.

Deakin and Graeff (1991) suggested that different 5-HT pathways and receptor subtypes modulate the neural substrates of depression, panic, and generalized anxiety. According to this assumption, the ascending 5-HT pathway that originates in the dorsal raphe nucleus (DRN), runs along the medial forebrain bundle, and innervates the amygdala and frontal cortex facilitates active escape or avoidance behaviors that occur in response to potential or distal threat (Blanchard *et al.*, 1989). These behavioral strategies rely on learning and, thus, relate to conditioned or anticipatory anxiety and, possibly, GAD. Postsynaptic 5-$HT_{2A/2C}$ and 5-HT_3, receptors are likely to be activated by this pathway. In turn, the DRN-periventricular pathway innervates the periventricular and periaqueductal gray matter. In these regions 5-HT inhibits inborn fight or flight reactions triggered by proximal danger (Blanchard *et al.*, 1989), acute pain, or asphyxia that may relate to panic disorder. This function of 5-HT is likely to be mediated by both $5HT_{2A/2C}$ and 5-HT_{1A} postsynaptic receptors.

Compounds representatives of three kinds of anxiolytics- namely the agonist of benzodiazepene receptor, diazepam; the 5-HT_{1A} partial agonist, buspirone; and the non selective 5-HT_2 antagonist, ritanserin have been shown to selectively impair inhibitory avoidance while leaving one-way escape unchanged (Graeff *et al.*, 1998). These results are compatible with the view that inhibitory avoidance relates to GAD. On the other hand impairment of open arm escape has been described with the chronic administration of the anti-panic compound, imipramine (Custodio Teixeira *et al.*, 2000). In our study, the results showed an anxiolytic effect in one of the tasks i.e inhibitory avoidance- in the Elevated T-

maze. Chronic administration of ME (30 and 100 mg/kg), EAF (10 and 30 mg/kg) and MF (30 mg/kg) insignificantly impaired inhibitory avoidance without affecting escape latency in animals pre-exposed to one of the open arms. Chronic administration of PE (50 and 100 mg/kg) and TF (10 and 30 mg/kg) significantly ($P<0.05$) impaired inhibitory avoidance without affecting escape latency in animals pre-exposed to one of the open arms. Chronic administration of PPE (10, 30 and 100 mg/kg) and KGE (10, 30 and 100mg/kg) significantly ($P<0.05$) impaired inhibitory avoidance without affecting escape latency in animals pre-exposed to one of the open arms. The results confirm with the previous data (Custodio Teixeira *et al.*, 2000) and suggest that pre-exposure provides a better index for escape. The extracts act in a way similar to compounds used in clinical practice to treat GAD,i.e., the benzodiazepine receptor agonist diazepam and the $5HT_{1A}$ partial agonist buspirone (Graeff *et al.,* 1993; Graeff *et al.*, 1998; Viana *et al.*,1994) and $5\text{-}HT_3$ antagonists. The classic anxiolytic diazepam impaired IA, but failed to change the escape latency. This finding corroborates with the previous finding in rats (Graeff *et al.*; 1993; Viana *et al.*, 1994), which demonstrated that diazepam selectively impaired IA, without influencing escape latency.

Chronically administered antidepressant drugs, particularly selective serotonin (5-HT) reuptake inhibitors (SSRIs), are clinically effective in the treatment of all anxiety disorders, while the clinical effectiveness of "traditional" anxiolytics, such as benzodiazepines (BDZs), is limited to generalised anxiety disorder or acute panic attacks. This implies that animal models of anxiety should be sensitive to SSRIs and other antidepressants in order to have predictive validity. It was also found that the effects of antidepressants in the so-called animal models of anxiety revealed anxiolytic-like action only in the isolation-induced calls in guinea-pig pups after acute administration. Some other models, such as marble-burying or conditioned-freezing behaviours, and isolation- or shock-induced ultrasonic vocalisation models, may detect anxiolytic-like activity of acutely administered antidepressants, although the sensitivity of these models is usually limited to SSRIs and other drugs affecting 5-HT uptake. The predictive validity of models of "anxiety", such as the plus-maze and light-dark transition tests or stress-induced hyperthermia, appears to be limited to BDZ-related drugs. Far less work has been done on chronic administration of antidepressants in animal anxiety models. Unless and until such studies have been undertaken, the true predictive value of the anxiety models will remain unknown (Franco *et al.*, 2002).

Thus, the present results show that fenugreek seeds, ginger rhizome and ginseng roots exert anxiolytic like effects in a specific subset of defensive behaviour, particularly those that have been related to GAD. The Indian variety of ginseng- *Panax pseudoginseng* showed similar anxiolytic profile when compared with its Korean counterpart- *Korean ginseng.*

The observation that 5-HT_{1A} receptor agonist 8-OH-DPAT caused a fall in blood pressure associated with sympathoinhibition and increased vagal drive to the heart led to the demonstration that central 5-hydroxytryptamine was involved in the control of the heart and vasculature. These effects are mediated by 5-$HT_{1/2/3}$ receptors. Vagal afferent evoked bradycardias (e.g. the cardiopulmonary reflex) are modulated by 5-HT_{1A} receptors located at the level of the parasympathetic nuclei. Also 5-HT_7 receptors have recently been shown to play a major role in the reflex activation of vagal bradycardia and this is believed at the level of nucleus tractus solitarius (NTS), the site of termination of cardiovascular afferents. Within the NTS, 5-HT_3 receptors are known to be activated by vagal afferents and cause the release of glutamate. Surprisingly 5-HT_{1A} and 5-HT_2 receptor mediated sympathoinhibition and excitation do not affect the outflow to the heart but only to the other organs. The central 5-HT containing neurons control the heart mainly through vagal outflow (Ramage, 2005).
Abnormalities of serotonergic system may play an important role in the pathophysiology of multiple cardiovascular disease states such as systemic hypertension, primary pulmonary hypertension and peripheral vascular disease (Frishman and Grewall, 2003). Further reports say that, *m*CPP increases catecholamine levels and stimulates sympathetic activity in rats. Activation of central 5-HT_{2C} receptors has stimulatory effects on adrenaline release (Chaouloff *et al.*, 1992). *m*-CPP induced increase in adrenaline levels are mediated by 5-HT_{2C} receptors (now may be 5-HT_{2B} receptors) (Bagdy *et al.*,1989). In the present study, ME and MF have antagonized the vasoconstrictive effects of *m*CPP and 5-HT in invasive blood pressure measurements in rats suggesting ME and MF of fenugreek seeds may possibly have 5-$HT_{2B/2C}$ receptor blocking effect. A similar effect was observed with Ketanserin (10 μg/kg). We also noted a significant fall in blood pressure of Adr (1μg/kg), NA (1μg/kg), PhE (1μg/kg) after treatment with PE (10 mg/kg) and TF (3 mg/kg). But surprisingly with TF (3 mg/kg) there was no significant fall in blood pressure with 5-HT (1 μg/kg). These

results were congruent with Ondansetron (1 mg/kg). With PPE (3 mg/kg) and KGE (3mg/kg) a significant fall in blood pressure was noted with Adr (1μg/kg) and 5-HT (1μg/kg).

The present study also investigated the antihypertensive property of methanolic extract (ME) of fenugreek seeds and its methanolic fraction (MF) in DOCA salt and fructose hypertensive rats. The study showed that chronic administration of ME (30 mg/kg/day; p.o.), MF (15 mg/kg/day; p.o.), PE (50 mg/kg/day; p.o), TF (10 mg/kg/day; p.o), PPE (30 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) for four weeks significantly reduced blood pressure in unilateral nephrectomized DOCA salt hypertensive rats. Chronic administration of ME (100 mg/kg/day; p.o.), PE (50 mg/kg/day; p.o.), PPE (30 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) for five to six weeks significantly reduced blood pressure in fructose hypertensive rats. Female rats were used in the DOCA salt hypertensive model as they appear to be more susceptible to develop hypertension (Balaraman *et al.,* 1989; Greenberg *et al.*, 1973).

The hypertensive mechanism due to chronic administration of DOCA-salt has been well documented. DOCA-salt being a mineralocorticoid causes salt and water retention and by this way contributes to the development of hypertension. Endothelin-1 (Matsumura *et al.*, 1999), atrial natriuretic peptides (Ogawa *et al.*, 1999), vasopressin (Bereck *et al.*, 1982) and 5-hydroxytryptamine (Dawson *et al.,* 1988; Balaraman *et al.*, 1989) are involved in the pathogenesis of this type of hypertension.

The 5 HT_{2B} receptor, first called atypical 5-HT receptor was originally described by Vane (Vane, 1957) as highly sensitive 5-HT receptor in the longitudinal smooth muscle of the rat stomach fundus. Activation the 5-HT_{2B} receptor can activate the extracellular signal – regulated kinases, members of the mitogen activated protein kinase family (Jean-Marie *et al.*, 1996). Since 5-HT is a vascular mitogen (Sanders and Bush, 1996) such an interaction holds obvious implications for the involvement of 5-HT_{2B} in the vascular growth often observed in hypertension. Hence 5-HT_{2B} antagonists may have a protective role in inhibiting vascular hypertrophy in hypertension. Recently it has been hypothesized that in hypertension that there is upregulation of 5-HT_{2B} receptor inorder to maintain an elevated blood pressure in rats made hypertensive by DOCA and NAME (Banes and Watts, 2002; Russel *et al.,* 2002). It has been previously reported that part of this increase in arterial sensitivity to 5-HT is due to a change in the receptor population that mediates contraction to 5-HT under conditions of

DOCA-salt hypertension. Specifically, Watts and colleagues (Watts, 1998; Watts *et al.*, 1995; Watts *et al.*, 1996) presented pharmacological and molecular evidence that a 5-HT_{2A} receptor population (ketanserin sensitive) primarily mediates contraction in arteries from normotensive rats, and a 5-HT_{2B} receptor population (relatively ketanserin insensitive) primarily mediates arterial contraction in DOCA-salt hypertension. This "switch" is important because 5-HT possesses 300-1,000 times higher affinity for the 5-HT_{2B} receptor compared with the 5-HT_{2A} receptor (Wainscott *et al.*, 1993; Wainscott *et al.*, 1996); thus lower concentrations of 5-HT are necessary to activate the 5-HT_{2B} receptor. Moreover, this finding makes important the reexamination of hypertension with new pharmacological tools that block the 5-HT_{2B} receptor, because the serotonergic antagonist most frequently tested has been ketanserin, and ketanserin possesses ~1,000 times lower affinity for the 5-HT_{2B} receptor compared with the 5-HT_{2A} receptor. LY-272015, a tetrahydro-β carboline – a recently developed 5-HT_{2B} receptor antagonist (Cohen *et al.*, 1996) has been shown to reduce blood pressure suggesting that 5-HT_{2B} receptors are endogenously up regulated in order to maintain an elevated blood pressure. Physiologically, this up regulation is important because 5-HT has a greater affinity for 5-HT_{2B} receptors as compared to 5-HT_{2A} receptors. Bilateral microinjection of 5-HT_3 receptor agonist, Phenylbiguanide (1.7-5 nmol) in nucleus tractus solitarius produced an increase in blood pressure and reduced the cardiovagal component of the baroreflex (Marahi and Laguzzi, 1995). The initial depressor response to Phenylbiguanide (PBG) is due to stimulation of known pulmonary chemoreceptors (including J receptors) whereas the pressor effect of phenylbiguanide is due to activation of receptors in the proximal arterial circulation (Giles and Sander, 1986).

On the basis of these findings we wished to test the putative 5-HT_{2B} antagonistic property of MF and ME and 5-HT_{2B} / 5-HT_3 antagonistic property of PE, TF, PPE or KGE in DOCA-salt hypertensive and fructose models. In DOCA-salt hypertensive rats, ME (30 mg/kg/day; p.o), MF (15 mg/kg/day; p.o), PE (50 mg/kg/day; p.o), TF (10 mg/kg/day; p.o), PPE (30 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o) were able to reduce blood pressure in DOCA-salt hypertensive rats but did not alter blood pressure in sham operated control rats implying an antihypertensive effect. Effects of ME and MF on vascular reactivity support that these act through influencing serotonergic pathway in hypertensive rats, as those selectively blocked the rise of serotonin in hypertensive rats, which is mediated by 5-HT_2 receptors.

Effects of PE and TF on vascular reactivity also support that these act through influencing serotonergic pathway in hypertensive rats, as those selectively blocked the rise of 5-HT and PBG in hypertensive rats, which is mediated by 5-HT_2 and 5-HT_3 receptors whereas PPE and KGE selectively blocked the rise of 5-HT in DOCA model.

Further, antihypertensive effect of above extracts and fractions does not involve modulation of the renin-angiotensin system since the antihypertensive effect was observed in DOCA-salt hypertensive rats, a low renin model of experimental hypertension.

Currently, researchers are taking interest in using a fructose induced hypertension model, as it gives the clue about the role of dietary changes in hypertension, which has became an important factor of modern life style.

The mechanism of fructose-induced hypertension is still not clear. Recent studies have shown that a high fructose diet is associated with increased blood pressure in rats (Bunnag *et al.*, 1997; Dimo *et al.*, 2001a; Dimo *et al.*, 2001b; Juann *et al.*, 1988). Several studies have demonstrated that chronic fructose feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia, hyperglycemia and hypertriglyceridemia in a relatively short time in normal rats (Erlich and Rosenthal, 1995; Hwang *et al.*, 1987; Zavaroni *et al.*, 1980). These metabolic changes lead to essential hypertension (Rosen *et al.*, 1997). Hyperinsulinemia could activate the sympathetic system, which in turn could elevate the BP (Hwang *et al.*, 1987). An impaired response to endothelium-dependent vasodilators in fructose fed rats has also been demonstrated (Richey *et al.*, 1998). A growing body of evidence indicates that locally generated vasoactive substances such as angiotensin-II (Ang-II) and nitric oxide (NO) are important determinants of the natural history of vascular disease. Recent evidence suggests that endothelial NO production could be decreased in fructose fed rats at both renal (Nagai *et al.*, 2002) and vascular levels (Shinozaki *et al.*, 1999). Alterations in both endothelial production of NO and VSMC growth could be associated with the initiation or progression of the atherosclerotic process and to vascular changes in hypertension. Korean red ginseng can improve the vascular endothelial dysfunction in patients with hypertension possibly through increasing the synthesis of nitric oxide (Sung *et al.*, 2000).

In the present study, the effect of the methanolic extract of fenugreek seeds ME (100 mg/kg/day; p.o.), PE (50 mg/kg/day; p.o.), PPE (30 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o.) were examined on hypertension induced by fructose (10%) given in

drinking water. Male rats were used in this study as female rats do not develop hypertension or hyperinsulinemia upon fructose feeding except after ovirectomy, suggesting that female sex hormones may confer protection against the effects of a fructose diet (Galipeau *et al.*, 2002). Parameters like Systolic blood pressure and vascular reactivity to 5-HT were modified significantly by chronic administration of ME (100 mg/kg/day; p.o.), PPE (30 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o.) in fructose hypertensive rats. Parameters like Systolic blood pressure and vascular reactivity to 5-HT, PBG were modified significantly by chronic administration of PE (50 mg/kg/day; p.o.) in fructose hypertensive rats. Moreover vascular reactivity to ACh was significantly altered by chronic administration of PPE (30 mg/kg/day; p.o) and KGE (30 mg/kg/day; p.o.) in fructose hypertensive rats.

Results from vascular reactivity also support the serotonin antagonistic property of ME. The blockade of rise of BP in hypertensive rats, which is due to 5-HT_{2B} receptor mediated, gives a clue that ME may act through 5-HT_{2B} receptors. Results from vascular reactivity also support the serotonin antagonistic property of PE, PPE and KGE. The inhibition of pressor effect due to 5-HT and PBG in hypertensive rats, which is due to 5-HT_{2B} and 5-HT_3 receptor mediated respectively gives a clue that PE may act through 5-HT_{2B} and 5-HT_3 receptors. The inhibition of pressor effect due to 5-HT in both the models supports the possible 5- HT_{2B} antagonistic activity of PPE and KGE. The significant potentiation of depressor response of ACh in fructose model by PPE and KGE may be possibly due to the NO production property of PPE and KGE (Sung *et al.,* 2000).

The sensitivity of rat fundus to 5-HT from fructose induced hypertensive rats was increased as compared to that in control rats. Treatment with ME (100 mg/kg/day; p.o.), PE (50 mg/kg/day; p.o.), PPE (30 mg/kg/day; p.o.) and KGE (30 mg/kg/day; p.o.) in fructose hypertensive rats shifted the dose response curve of 5-HT significantly to the right compared to fructose hypertensive rats suggesting upregulated 5-HT_{2B} receptors may be blocked. Similarly treatment with ME (100 mg/kg/day; p.o.) in control rats also shifted the dose response curve of 5-HT towards the right indicating 5-HT_{2B} antagonistic properties of the extract. The nature of antagonism initially for the first few doses appeared to be of competitive type but at higher doses the nature of antagonism shifted to the non- competitive type.

The data of our study suggest that ME of fenugreek seeds, PE of ginger, PPE and KGE can play a major role in elucidating the mechanisms underlying the pathogenesis of fructose-fed hypertension as demonstrated by the beneficial effects on blood pressure.

Collectively, the results of our study suggest that 5-HT plays an important role in development of hypertension and the antihypertensive activity of methanolic extract and methanolic fraction of *Trigonella foenum graecum* seeds may be partly due to 5-HT_{2B} receptor antagonism and the antihypertensive activity of pet ether extract and toluene fraction of *Zingiber officinale* may be due to 5-HT_{2B} /5-HT_3 receptor antagonism while; *Panax pseudoginseng* extract and *Korean ginseng* extract may be due to 5-HT_{2B} antagonism and NO production. The Indian variety of ginseng- *Panax pseudoginseng* showed similar antihypertensive actions when compared with its Korean counterpart- *Korean ginseng*.

The involvement of HPA (ACTH/CRF) in the modulation of antihypertensive effects of the above extracts has been ruled out by testing the effect of extracts in bilateral adrenolectomised rats. There was no change in response of the extracts when compared to the control animals (Zeirer, 1991).

Various studies have shown that 5-HT levels are high in streptozotocin STZ rats (Martin *et al.*, 1995). 5-HT produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT_3 receptors (Goyal *et al.*, 2003). It has been further shown that chronic treatment with 5-HT_2 antagonist sarpogrelate and 5-HT_3 antagonist ondansetron produce number of beneficial effects in diabetic rats (Goyal *et al.*, 2003). 5-HT receptor stimulation causes an inhibitory effect on secretion of insulin (Srivastava, 1984a).

The GCMS data of *Z. officinale* shows the presence of gingerol (nearly 15%). This constituent has a 5-HT_3 antagonistic activity. Ginger exerts its central anti-emetic effect via 5-HT_3 antagonism (Lumb, 1993). Galanolactone, a triterpenoid and gingerols a pungent principle isolated from ginger are reported to be competitive antagonists predominantly at 5-HT_3 receptors (Huang *et al.*, 1991). The LCMS data showed the presence of ginsenoside Rb1 in PPE indicating the presence of the active constituent mediating anxiolytic and

antihypertensive effects. The HPTLC fingerprinting has showed that the active fraction isolated from the crude drug was proportionately in significant concentration.

Fenugreek contains small amounts of coumarins, chemicals that are used in drugs to increase the time blood needs to clot (Hoult, 1996). It is found that fenugreek, ginger and ginseng extracts inhibited platelet aggregation as it significantly prolonged the clotting time *in vitro* (Teng *et al.*, 1989). These actions can also be correlated to 5-HT receptors, which are involved in platelet aggregation (Srivastava, 1984b).

We observed that ME, PPE and KGE reduced lithium induced head twitches, a behaviour which is mediated by postsynaptic 5-HT_2 receptor mechanisms.

In haloperidol-induced catalepsy, the extracts at the selected doses had no effect on catalepsy. These changes can be correlated to decrease in brain DA concentration which inturn proves that the effects of locomotor activity is purely due to its treatment effects. PPE and KGE significantly potentiated pentobarbital induced sleep time. This can be correlated to increased GABAergic transmission, a possible mechanism for anxiolytic effect in addition to its 5-HT_2 receptor antagonistic effects.

In all the studies conducted with ginseng extracts, PPE showed effects similar to KGE indicating the presence of similar constituents in the Indian and Korean variety. Cultivation of Indian variety of ginseng for commercial value under controlled conditions (to increase yield) is therefore recommended.

In conclusion, our data collectively suggests that *Trigonella foenum graecum, Zingiber officinale, Panax pseudoginseng* and *Korean ginseng* possess antihypertensive and anxiolytic activity. The mechanism of action could be due to the involvement of 5-HT receptors. However further studies like in-*vivo* dialysis or radioligand-binding assays may prove this hypothesis true.