8. SUMMARY AND CONCLUSION

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. It is defined as a feeling of uncertainty, apprehension or tension. The heightened level arousal and subjective feeling of fear is the feature of all major categories of anxiety disorder. It is regarded as a particular form of behavioral inhibition that occurs in response to environmental events. Human anxiety disorders are broadly grouped according to symptomatology and responsiveness to pharmacological and psychological treatments. Generalized anxiety disorder is a chronic disorder characterized by excessive long-lasting anxiety and worry about nonspecific life events, objects and situations. The complexities of the central nervous system make diagnose, treatment and amelioration of these debilitating illnesses exceptionally difficult.

Advancement in this area would be invaluable contributions in the effort to reduce the global impact of anxiety-based conditions. Postulations have implicated deregulations of specific neurotransmitters such as serotonin, dopamine and gamma-amino butyric acid (GABA) as potential causes for both depression and anxiety disorders. The universality of herbal remedies in many cultures makes them an appropriate treatment to explore. To keep this ever-increasing trend alive, it is imperative to obtain and maintain the herbal raw materials and finished products. Therefore, WHO has evolved internationally recognized guidelines to support the policies on herbal drugs to study their potential usefulness including evaluation, purity, safety and efficacy. Various
aspects on anxiety and its impact in various diseases have been described in Chapter 1.

In the light of the above points, the present study was mainly focused on Phytochemical and Pharmacological evaluation of leaves of *F.indica* Linn for anti-anxiety activity with objectives of preparation of extracts, identification, isolation, characterization of compounds, performance of acute oral toxicity and anti-anxiety activity from the leaves of the plant. Aim and objective and flow chart of work plan was described in chapter 2 and chapter 3.

*F.indica* is an indigenous medicinal plant widely distributed in Bangladesh and India. In India, *F.indica* is found from Punjab to Bihar, the Deccan and the Southern Peninsula. *F.indica* is a small deciduous thorny shrub, found throughout India in scrub forests and rocky hills up to 900m. The roots are used in treating poisonous bites, skin diseases, nephropathy and psychopathy. The leaves are used for treating pruritus and scabies. Fruits are useful for treating jaundice, gastropathy and splenomegaly. A complete review on phytochemical and pharmacological profiles of the *F.indica* was discussed in Chapter 4. This provides updated information on the therapeutic potential, botanical identity and traditional aspects of their use.

Chapter 5 describes the various materials and methods used for phytochemical and pharmacological investigations. The leaves of the plant (500 g) were shade dried and powdered. The powdered material was extracted using
Soxhlet apparatus by continuous hot extraction process successively with petroleum ether, chloroform and ethanol for 24 hrs.

The Preliminary Phytochemical evaluation of ethanolic extract of *F.indica* revealed the presence of alkaloids, steroids, flavonoids, phenolic compounds, tannins, proteins, amino acids, glycosides and sugars. So the alcoholic extract was selected for further analysis.

Ethanol extract was subjected to column chromatography for the isolation of phytoconstituents and eluted with solvents of increasing polarity. The fractions so obtained were analyzed by TLC. Fractions having same Rf value were pooled. As a result two fractions A and B were obtained. Concentration of fraction A eluted with chloroform: acetone (6:4) yielded 2 compounds (yellow color powder and yellow color needles). Similarly concentration of fraction B eluted with Ethyl acetate: Butanol (6:4) yielded 3rd compound, which was also yellow in color. The compounds were then purified and subjected for characterization by IR, NMR and MASS spectrometry. The yield was found to be 17 mg, 15 mg and 12 mg for Compound 1, Compound 2 and Compound 3 respectively.

The melting point, IR and NMR spectra of isolated compounds 1,2 and 3 exhibited identical spectra when compared to that of existing data’s of 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-chromen-4-one (Fisetin); 6-((E)-4-hydroxy-3-methylbut-2-enolox)-2-phenylchroman-2,7-diol and 3,5,7,4’-tetra hydroxy flavone, (kaempferol). The mass spectrum of isolated compounds 1, 2 and 3 showed molecular ion peaks at m/z 286.2, 342.39 and 286, which corresponds to
the above mentioned compounds. These compounds **Fisetin, 6-((E)-4-hydroxy-3-methylbut-2-enoloxy)-2-phenylchroman-2,7-diol** and **Kaempferol** were isolated for the first time in this plant.

Acute oral toxicity study was performed according to the OECD guideline 423 - Acute toxic class method. Female Sprague dawley rats of 160-180 g were used for the study. The animals were grouped into two (control and test group) of 3 animals/group. The test drug was administered once orally via gastric intubation at a dose level of 2000mg/kg body weight. Lethality, abnormal clinical signs and body weight changes were observed on the day of dosing and thereafter for 13 days. Gross pathological changes were also observed on the day of termination. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. According to Organization for Economic Co-operation and Development (OECD) guidelines for acute oral toxicity, an LD$_{50}$ dose of 2000 mg/kg and above is characterized as unclassified and hence the drug is found to be safe.

Anti-anxiety activity was carried out using the various methods like Elevated plus-maze test, Hole-Board (Head dipping test), Staircase exploration, Light-Dark exploration, Open-Field test and Social Interaction test.

The animals were administered with their respective drugs for 5 consecutive days. On day 5, 1 h after drug administration, mice were individually placed in the center of the maze facing open arm and allowed to explore for 5 min. The number of entries in to open and closed arms and the total time spent in both
arms were recorded for Elevated plus-maze test and the immobility period, number of head dips and number of rearing were recorded for Hole-Board (Head Dipping) method. Total numbers of steps climbed and total no of rearing were recorded for a period of 3 min for staircase exploration. In light-dark exploration methods, mice were placed individually in the illuminated part of the cage and total number of crossings, number of crossings between the light and dark area, total time spent in illuminated and dark part of the cage, number of rearing in the illuminated and dark part of the cage and number of defecation units were recorded for a period of 5 min.

In open-field test, the entire room except the open field was kept dark. Drugs were administered for 3 consecutive days and on day 3, 1 h after the last drug administration, each animal was centrally placed in the test apparatus for 5 min and ambulation, rearing, self grooming, activity in centre and numbers of fecal droppings were recorded for open field test.

In social interaction test, the animals were housed individually for 5 days before testing. On day 6, the mice were placed individually in the box and were given two 7.5 min familiarization sessions at 2 h interval. On day 7, 1 h after drug administration mice were paired on weight basis and placed in the box for 7.5 min. During this time total time spent by the mice pair in “social interaction” including sniffing, following, grooming, kicking, boxing, licking, crawling under or over the partner were recorded. Data were expressed in mean ±SEM. Mean difference between groups were analyzed by One way ANOVA. P<0.05 was considered significant.
Chapter 6 gives information about the results of phytochemical and pharmacological investigations. Anxiolytics increase the time spent and number of entries in open arm. Ethanolic extract of *F.indica* at doses of 100mg/kg and 200mg/kg (Test doses) also significantly increased number of entries and time spent in open arm which clearly indicates the anxiety has been considerably reduced in elevated plus-maze.

In hole-board (head dipping) test doses significantly increased head dips and rearing whereas immobility period got reduced compared to that of Diazepam. Both the test doses showed decreased number of rearing and increased number of steps climbed compared to that of Diazepam in Staircase Test. All the parameters showed significant anxiolytic activity except for number of defecation units which is insignificant in light-dark transition test.

In Open-field test, mice treated with test dose levels showed dose dependent significant increase in Open-field ambulation, rearing, self grooming and activity in centre compared to that of control. Further, mice treated with test doses spent significantly more time in social interaction in comparison to control and this anxiolytic activity is found to be dose dependent.

From the above study it may be concluded that evaluation of anti-anxiety activity of *F.indica* along with phytochemical evaluation has given encouraging results and the compounds obtained may be the reason for anti-anxiety activity. So the present study to provide scientific validation for anti-anxiety activity has accomplished its purpose.
Further investigation will hopefully pin point the exact mechanism of action of extract and isolated compound for its anti-anxiety activity. So that, in near future these can be used as therapeutic agents after clinical trials.