Development of Economic Herbal Based Drug Substitute from *Citrus paradisi* (Grape fruit) for Existing Anti-anxiety Drug Modules

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**Abstract**

Persistent and unrelenting stress often leads to anxiety and unhealthy behavior. Benzodiazepines are used as a first line of treatment however difficulties with pharmacotherapy of anxiety disorders such as dependence and low response rate encourage researchers to find new approaches. A number of studies have been done on anti-anxiety activity of medicinal plants but major constraint is non suitability of the tested plant material for human use and non availability of plant materials in bulk at economical rates. This study entails to development of safe anti-anxiety economic drug of easy availability. In normal course aromatic oils from plant are being used however authors selected four varieties of plant *Citrus paradisi* available worldwide and tested the anti-anxiety activity of leaf extracts as leaf extracts can be made available at commercial scale. All the selected varieties have demonstrated a potential diazepam like effect in methanolic extracts at a dose of 100 mg/kg body weight using elevated plus maze model. The results strongly justify the use of *Citrus paradisi* leaf extracts for treatment of anxiety. Further studies are in progress to find out active component involved in the anti-anxiety effect.

**Keywords:** Anxiety; *Citrus paradisi*; Diazepam; Elevated plus maze

**Introduction**

Stress and anxiety are common psychiatric manifestations of the modern world and lifestyles. In small quantities, stress and anxiety are good; they can motivate and help one be more productive. However, too much stress, or a strong response to stress, is harmful. It can set up for general poor health as well as specific physical or psychological illnesses like infection, heart disease, or depression. Persistent and unrelenting stress often leads to anxiety and unhealthy behaviors. Anxiety is a Central Nervous System disorder with emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat [1,2].

**Therapies available and limitations**

Benzodiazepines are used as a first line of treatment. Today, at least 20 million people worldwide are prescribed these “minor tranquilizers.” Meanwhile, Western European and North American countries are facing epidemic levels of citizens hooked on these drugs. While benzodiazepines are a disaster in terms of the public health, pharmaceutical companies rake in a whopping $21 (€ 14 billion) billion a year selling them. So, difficulties with pharmacotherapy of anxiety disorders such as dependence and low response rate encourage researchers to find new approaches [3].

**Alternative therapies**

From the past, the role of medicinal plants has been a subject of intense interest. A number of studies have been done on anti-anxiety activity of medicinal plants but major constraint towards development of a marketable formulation is non suitability of the tested plant material for human use and non availability of plant materials in bulk at economical rates. This study entails to development of safe anti-anxiety economic drug of easy availability. In normal course aromatic oils from plant are being used for antianxiety activity but the cost of aromatic oils and its availability and cumbersome administration procedure creates a limitation for a market product. At the same time aromatic oils can be used topically or for inhalation purposes whereas leaf extracts can be a better medium for formulation development which can be easily administered.

**Materials and Methods**

**Plant material**

The leaves of *Citrus paradisi* of four different varieties i.e. duncan, foster, marshseedless and star ruby were procured from a identified and cultivated source: Punjab Agricultural University, Regional Centre, Abohar (Punjab, India) in the month of March-April, 2013.

**Preparation of extracts**

Leaves of different varieties were dried in shade and powdered. The powdered leaves (100 g) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60-
Test animals

The experimental animals [Swiss albino mice (20-30 g) of either sex] were procured from the approved animal House, Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur. (IAEC No. ATRC/05/13). The animals were given standard laboratory feed and water ad libitum. The experiments were performed between 6.00 am to 11.00 am. The experiments were conducted in a semi-sound dryness on the water-bath.

Anti-anxiety activity

Animals were divided into six (I-VI) groups for each variety of source plant.

For each variety

1. Group I was a negative control and administered only vehicle (consisting of simple syrup IP and carboxy methyl cellulose (5%).
2. Group II was a positive control and was given standard drug, diazepam (2 mg/kg, orally), suspended in the vehicle.
3. Group III-VI were treated as test groups and were given petroleum ether (60–80°C), chloroform, methanol and water extracts of different varieties from the leaves of Citrus paradisi at different doses viz. 50, 100, 200 and 400 mg/kg body weight respectively. Mice were treated orally with all the test solutions, standard drug and control for 5 days once daily at a stipulated time and last dose was given on the 5th day, 45 minutes prior to study.

Elevated plus maze model (EPM)

The elevated plus-maze model is well established animal model for testing anxiolytic drugs. The elevated plus-maze apparatus consist of two open arms (16×5 cm for mice and 50×10 cm for rats), two closed arms (16×5×12 cm for mice and 50×10×40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor. The animals were placed individually in the centre of the maze, head facing towards open arms [11,12].

Statistical analysis

The anxiolytic activities of the extracts, diazepam and control were analyzed by one-way analysis of variance (ANOVA). The test groups were compared with standard/control by Dunnett Multiple Range Test. Difference were considered significant at p<0.05.

Results and Discussion

Acute toxicity study

Acute oral toxicity studies revealed the non-toxic nature of different extracts of different varieties of Citrus paradisi. There was no morbidity observed or any profound toxic reactions found at a dose of up to 2000 mg/kg body weight, which indirectly reflects the safety profile of the plant extract.

Anti-anxiety activity

The results obtained from the EPM model, indicate that methanolic extract of all the varieties showed significant (p<0.05) anti anxiety activity as compared to control and was almost at par with standard drug diazepam. At 50 mg/kg of body weight there was an increase in the time spent in open arms when the mice were administered leaf extracts from all the varieties. The average time spent in open arms further increased from 7.987 ± 0.613 (sec) in control to 21.750 ± 0.243 (sec) in methanolic extract at a dose of 100 mg/kg of Citrus paradisi var. duncan, from 10.023 ± 0.850 (sec) in control to 25.050 ± 0.369 (sec) Citrus paradisi var. marsheedless and from 8.642 ± 0.351 (sec) in control to 20.948 ± 0.651 (sec) in Citrus paradisi var. staraby. At a higher dose of 200 mg/kg the leaf extracts demonstrated
This effect has been attributed to the affinity of Passiflora coerulea been described in many plant species used in folk medicine such as of Parkinson’s patients. Flavonoids with anxiolytic activity have or B, thereby working as anti-depressants or to improve the conditions Flavonoids of several classes are inhibitors of monoamine oxidase A receptor resulting in sedation, anxiolytic or anti-convulsive effects. CNS several flavones bind to the benzodiazepine site on the GABA The extracts from the plant shows the presence of flavonoids and the Citrus paradisi may be related to their flavonoid content. anxiolytic effects. The anxiolytic effects of methanolic extract of four (less than 100 mg/kg) of the plant extract did not show any significant of significant anxiolytic effects at doses higher than 100 mg/kg could the plant extract was more prominent at 100 mg/kg and doses higher or lower than this did not show a consistent anxiolytic effects. The lack the active component involved in the anti-anxiety effect. Synthetic drugs and medications possess enormous side effects whereas toxicity studies of our proposed extract have proven to be safe, so these herbs with a wide therapeutic applicability promise to alleviate anxiety with very few adverse effects.

**Conclusion**

There is a paradigm shift towards use of herbal remedies or herbal based formulations. The role of medicinal plants in disease prevention and treatment has always been remarkable. This study was conducted with an aim towards develop of safe anti-anxiety economic drug of easy availability. In normal course aromatic oils from plant are being used however authors selected four varieties of plant Citrus paradisi available worldwide and tested the anti-anxiety activity of leaf extracts as leaf extracts can be made available at commercial scale. All the selected varieties have demonstrated a potential diazepam like effect that strongly justify the use of Citrus paradisi leaf extracts for treatment of anxiety in human trials. Further studies are in progress to find out active component involved in the anti-anxiety effect. Synthetic drugs based formulations. The role of medicinal plants in disease prevention and treatment has always been remarkable. This study was conducted with an aim towards develop of safe anti-anxiety economic drug of easy availability. In normal course aromatic oils from plant are being used however authors selected four varieties of plant Citrus paradisi available worldwide and tested the anti-anxiety activity of leaf extracts as leaf extracts can be made available at commercial scale. All the selected varieties have demonstrated a potential diazepam like effect that strongly justify the use of Citrus paradisi leaf extracts for treatment of anxiety in human trials. Further studies are in progress to find out active component involved in the anti-anxiety effect. Synthetic drugs and medications possess enormous side effects whereas toxicity studies of our proposed extract have proven to be safe, so these herbs with a wide therapeutic applicability promise to alleviate anxiety with very few adverse effects.

**References**


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<th>Group</th>
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<tr>
<td></td>
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<td>Petroleum ether (Mean ± SEM)</td>
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<tr>
<td>I</td>
<td>Vehicle</td>
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<tr>
<td>II</td>
<td>Diazepam</td>
<td>-</td>
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<tr>
<td>III</td>
<td>50 mg/kg</td>
<td>13.165 ± 0.568</td>
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<td>IV</td>
<td>100 mg/kg</td>
<td>16.690 ± 0.461</td>
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<td>V</td>
<td>200 mg/kg</td>
<td>13.728 ± 0.541</td>
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<tr>
<td>VI</td>
<td>400 mg/kg</td>
<td>11.003 ± 0.414</td>
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Values are Mean ± SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control

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<td>7.953 ± 0.400</td>
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Values are Mean ± SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control

**Table 3: Anti-anxiety activity of various extracts of leaves of Citrus paradisi var. marshseedless using EPM.**

**Table 4: Anti-anxiety activity of various extracts of leaves of Citrus paradisi var. starruby using EPM.**
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