COMPARATIVE EVALUATION OF ANXIOLYTIC EFFECTS OF PURE LEMON JUICE VERSUS RECONSTITUTED LEMON DRINK

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ABSTRACT

Background: Anxiety is one of the most common complain suffered by majority of population in response to stress, apprehension and various other environmental factors. It is behavioral mechanism to cope with difficult situations. In past few years use of anxiolytic drugs have increased a lot however herbal remedy is safer, cheaper and easily available, so nowadays there is more focus on screening nutrients used in everyday life. Objective: The current study is based on evaluating the anxiolytic effects of pure lemon juice and lemon drink reconstituted from powdered forms available in market. Methodology: The present study has been conducted on albino mice of either sex weighing between 18-24gm. The mice were equally divided into 4 groups (control, Standard, pure lemon juice and reconstituted lemon drink). Head dip method was used for evaluating the anxiety. Result: Our study revealed pure lemon juice had marked anxiolytic activity (24 head dips) in comparison to control (50 head dips) and standard drug lorazepam (22 head dips) where as reconstituted lemon drink had mild anxiolytic effect (32 head dips). Conclusion: From above study we can conclude that lemon possesses essential oils and flavanoids due to which anxiolytic effect is observed. As non-pharmacological therapy lemon juice can be incorporated in diet of people who suffer from anxiety. Since it is a drink commonly used in every day life it will not have any harmful effects and will be better than allopathic anxiolytic drugs which have number of adverse effects associated with them.

KEYWORDS: Anxiolytic, lemon juice, reconstituted drink, Herbal.
INTRODUCTION
Anxiety is a state of nervousness, worry, an unpleasant state of dread over events that have not occurred.\(^1\) Anxiety can result from stress, fear of things (phobias), environmental factors, depressive disorders,\(^2\) and diseased conditions.\(^3\) There are various forms of anxiety such as generalized anxiety disorder, social anxiety disorder, panic disorder and bipolar disorder.\(^4\) When an individual is in a state of anxiety he undergoes four types of experiences i.e. physical tension, mental apprehension, dissociative anxiety and physical symptoms.\(^5\) Neurotransmitter dysregulation is a major cause of anxiety. Gama aminobutyric acid (GABA) is an inhibitory neurotransmitter; its reduced level leads to development of anxiety.\(^6\) Another theory suggests that the amygdala, which is the center of processing of fear and emotions, its neuronal circulation might be disrupted in anxiety.\(^7\) The Zulf Self Rating Anxiety Scale or Taylor Manifest Anxiety Scale is a standardized clinical questionnaire for the detection of anxiety based on symptoms.\(^8\) Presently, commonly used medications for the relief of anxiety include Selective Serotonin Reuptake Inhibitors (SSRI), Monoamine oxidase Inhibitor (MAOI) and Pregablin etc.\(^9\)

Multiple approaches are available for treatment of different ailments. Plant material has been used since ancient civilization for the treatment of various diseases.\(^10\) Citrus limon belonging to the family Rutaceae is also known as lemon. It is commonly cultivated in South Asian countries, USA, Italy, Turkey, Pakistan, India and Australia etc.\(^11\) It is widely used in daily life for multiple purposes including reduction of weight, neurotonic, mood enhancer and as an antibacterial.\(^12\)

Lemon is a very good dietary supplement. It is a rich source of vitamins containing Vitamin C, Vitamin B (Thiamine, Riboflavin, Pyridoxine, Pantothenic acid, and folate). It also contains Trace elements like Calcium, Magnesium, Zinc, Iron, and Potassium.\(^13\) Its main chemical constituents include Flavanoids, Polysaccharides, sugars, volatile compounds, carotenoids, and organic acids.\(^14\)

According to recent study positive behavioral effects, memory enhancing, and antidepressant effects of Citrus Limon essential oil and juice have been found in rats.\(^15\) The present study is designed to evaluate the anxiolytic effects of lemon juice in mice at different dose and to compare the anxiolytic effect of Citrus limon juice in mice with powdered lemon drinks available in market which are reconstituted for use.
MATERIALS AND METHOD

Experimental Animals: The study was carried out on albino mice of either sex weighing between 18-24gm. They were bred and housed in animal house of Jinnah University for Women. The animals were given food and water ad libitum. They were kept under constant environmental conditions of 23± 2°C.

Material: Fresh lemons were purchased from local market in Karachi and were identified by Department of Pharmacognosy, Faculty of Pharmacy Jinnah University for Women. The fresh lemons were squeezed by hand and then juice was filtered.

The Lemon powder was also purchased from local market in Karachi. It was reconstituted in distilled water by preparing solution 25mg/5ml.

Dosing Regime: The mice were equally divided into 4 groups (control, Standard, pure lemon juice and reconstituted lemon drink). The control mice were given distilled water 0.8ml, Standard taken was Lorazepam 2mg/60kg that means 0.3mg/kg, this dose was adjusted according to weight of mice in milligrams. Stock solution was prepared 12mg/60ml in distilled water and dose was administered by serial dilution method orally.

Pure lemon juice was given 0.8ml and lemon powder was given 150mg/kg.\textsuperscript{[16]} this was reconstituted as 25mg/5ml and dose was given by serial dilution method orally. All the groups were given dose once daily. The study was carried out for 7 days.

Head Dip Test: The anxiety effect in Rodents (mice) was observed by Hole Board or Head Dip Test. The apparatus consists of an enclosed wooden rectangular box (35cm×45cm×45cm). The holes are 2.5cm in diameter and found in all walls.\textsuperscript{[17]}

Procedure: Half an hour before starting the experiment the animals were familiarized with the environment in which the Hole Board apparatus was placed. The temperature was kept constant (same as in animal house). The mice that were ignorant of the apparatus were placed in the centre area and allowed to freely explore for 5 minutes. The number of times the mouse stuck out its snout was noted. After each observation the apparatus was cleaned with 70% alcohol to clear whiff of previous subject.\textsuperscript{[18]}
RESULT

By Alcarz and Jimenez (1989) method all statistical procedures are performed. By taking mean of all the values they are compared with means of control and standard drug and by student significance t-test the significance of difference between means are determined. A value of \( p< 0.05 \) is considered significant, \( p< 0.001 \) as more significant and \( p< 0.0001 \) as highly significant.

Table 1 and Graph 1 shows the effect of Water (Control), Lorazepam (Standard), Natural Lemon Juice and Reconstituted Lemon Drink on Head Dip activity in mice. Post-hoc analysis by Newman-keuls test showed that the number of head dips decreased highly significantly \((<0.0001)\) with Natural Lemon Juice and Reconstituted Lemon Drink after 7 day dosing.

The number of head dips in control were 50, while after dosing with Natural Lemon juice the head dips reduced to 24. The number of head dips after dosing with Reconstituted Lemon drink was 32. The head dips observed after dosing with Lorazepam were 22.

Effect of Lemon Juice on Head dip activity (7 days).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Head dips</th>
<th>p-value (control)</th>
<th>p-value (standard)</th>
<th>p-value (between drugs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>50 ± 1.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>22.0 ± 1.79</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pure Lemon Juice</td>
<td>24.90 ± 1.52</td>
<td>***0.000</td>
<td>IS 0.4</td>
<td>***0.000</td>
</tr>
<tr>
<td>Reconstituted Lemon Drink</td>
<td>32.4 ± 1.17</td>
<td>***0.000</td>
<td>***0.000</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D.

N=10= number of animals.

***p<0.0001 = highly significant.

IS = insignificant difference.
DISCUSSION
Herbal preparations have been used since ancient times because of their efficacy, safety and low cost. Nowadays research is being focused on fruits, vegetables and juices inorder to incorporate them properly in diet and overcome symptoms of disease condition.

On initial exposure to apparatus, the animal tries to find an escape route due to fearful and neophobic response.\cite{19} Stressful condition of animal is further confirmed by elevated levels of corticosteroids in adult rats following first exposure to apparatus.\cite{20} If it is assumed that on exposure to apparatus, anxiety develops due to state of fear so decrease in number of dips shows relieve from anxiety or reduced fear.\cite{21} This postulation supports our above results that lemon juice and reconstituted lemon drink possess anxiolytic effect.

Flavanoids are the main components present in lemon responsible for most of the medicinal properties.\cite{22} Flavanones a type of flavanoid is present in citrus fruits either as glycoside type or aglycone type. Naringenin and hesperetin are important aglycone type of flavanones while neohesperidosides and rutinosides are important flavanones of glycone type. Flavanoids possess a number of beneficial effects such as anti-mutagenic, anti-poliferative, anti-inflammatory, anti-oxidant and also possesses anti-depressant effect.\cite{23}

It is a known fact that majority of anxiolytic drugs act by mediating effect on GABA neurotransmitter by binding to GABA\textsubscript{A} receptor and potentiating gabanergic inhibition in CNS by opening chloride channels and causing hyperpolarization which reduces firing rate of critical neurons in brain or drug directly activates GABA receptor.\cite{24} Monoterpenes is another important constituent of Citrus limon and it is thought to possess anxiolytic effect by Gabanergic mechanism.\cite{25} Besides this the antioxidant effect produced by flavanoid component is also thought to provide neuroprotective effect in mice hippocampus.\cite{26}

The standard drug taken was lorazepam an intermediate acting benzodiazepine which possesses anxiolytic effect at low doses and hypnotic effect at high doses.\cite{27} The mechanism of action is by potentiating gabanergic effect. Our results show very similar effect of natural lemon juice to that of lorazepam. The reconstituted lemon drink also showed positive effects but lower than natural lemon juice probably because of dilution factor (addition of water) or presence of other constituents i.e preservatives.
From the above study we can conclude that Natural lemon juice and reconstituted lemon drinks use should be incorporated in daily diet of people who suffer from anxiety and depression. Besides that it can be taken by normal people too since it will help reduce stress and produce positive effect on memory.

REFERENCES
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