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## Evaluation of Anxiolytic Effect of Pineapple Juice

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### Research Article

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### ABSTRACT

**Background:** Nowadays due to increasing demands of our society's lifestyle stress, worry and feeling of un satisfaction has increased a lot leading to anxiety which has become one of the most common problem faced by population. In past few years use of anxiolytic drugs have increased a lot however because of adverse effects faced by patients, increased cost and dependence nowadays there is more focus on screening nutrients used in everyday life.

**Objective:** The current study is based on evaluating the effects of Pineapple juice in anxiety.

**Methodology:** The present study has been conducted on albino mice of either sex weighing between 18-24 gm. The mice were equally divided into 3 groups (control, Standard and Pineapple juice). Head dip, light and Dark model, Open Field and elevated plus maze methods were used for evaluating anxiety.

**Result:** Our study revealed Pineapple Juice had significant

anxiolytic activity in comparison to control and standard drug lorazepam.

**Conclusion:** From above study we can conclude that Pineapple possesses Vitamins and flavonoids due to which anxiolytic effect is observed. As non-pharmacological therapy Pineapple juice can be incorporated in diet of people who suffer from anxiety. Since it is a tasty drink that is commonly consumed in everyday 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.

## INTRODUCTION

Anxiety is defined as condition in which person has unpleasant feelings of dread over events that have not occurred, there is state of nervousness along with useless worry over things <sup>[1]</sup>. When an individual is in state of anxiety he undergoes four types of experiences i.e. physical tension, mental apprehension, dissociative anxiety and physical symptoms <sup>[2]</sup>. Anxiety not only affects brain or nervous system it affects the whole human body. Common symptoms of anxiety faced by patients are feeling of being frightened, restlessness, breathing problem, panic, Insomnia, nervousness, worry, headache and backache etc <sup>[3]</sup>. Causes of anxiety are usually stress, fear of things (phobias), environmental factors, depressive disorders <sup>[4]</sup> and diseased conditions <sup>[5]</sup>. Anxiety is classified into various types such as generalized anxiety disorder, social anxiety disorder, panic disorder and bipolar disorder <sup>[6]</sup>.

Another cause of anxiety is thought to be neurotransmitter dysregulation. Reduced levels of inhibitory neurotransmitters especially GABA (Gama amino butyric acid) can lead to anxiety <sup>[7]</sup>. Another cause that leads to anxiety might be disruption of neuronal circulation in amygdala which is center of processing of fear and emotions <sup>[8]</sup>. Zulf Self Rating Anxiety Scale or Taylor Manifest Anxiety Scale is standardized clinical questionnaires for detection of anxiety based on symptoms <sup>[9]</sup>. Presently commonly used medications for relieve of anxiety include Selective Serotonin Reuptake Inhibitors (SSRI), Monoamine oxidase Inhibitor (MAOI) and Pregablin etc <sup>[10]</sup>.

Nutrients were used traditionally not only as source of food but also because of their beneficial effects on health. Later as allopathic medicine developed their use was declined <sup>[11]</sup>. Nowadays however due to better availability, safety profile and cost effectiveness the research is being focused on nutrients used in everyday life.

Ananas comosus commonly known as pineapple is a tropical fruit belonging to family Bromeliaceous <sup>[12]</sup>. Pineapple is perennial and herbaceous plant. It is most commonly cultivated in Brazil, Thailand and Philippines <sup>[13]</sup>. It is not only delicious fruit but is also rich in nutrients. Raw pineapple contains 13.12 gm carbohydrates (including sugar and dietary fiber), 0.12 gm Fat and 0.54 gm of Protein. It provides 209 kJ of energy <sup>[14]</sup>. It contains vitamins such as Vitamin

C, Thiamine, Riboflavin, Niacin, Pantothenic acid; Folate and Vitamin B6. Besides this it also contains trace metals such as Calcium, Iron, Manganese, Magnesium, Potassium, Sodium and Zinc [15].

Its main chemical constituents include Flavonoids, Phenols, and Polysaccharides, sugars, volatile compounds, carotenoids and Bromelain [16]. Pineapple possess number of health benefits such as reduces risk of asthma [17], Reduces Blood pressure [18], has anticancer properties [19], healing and anti-inflammatory effects [20]. During Pregnancy use of pineapple juice during first trimester is contraindicated as it can increase uterine contractions leading to termination of pregnancy [21]. Side effects experienced include vomiting, nausea, diarrhea, skin rash, excessive menstrual flow and swelling of mouth and cheeks on excessive consumption [22].

The present study is designed to evaluate the anxiolytic effects of Pineapple juice in mice.

## MATERIALS AND METHODS

### Experimental Animals

The study was carried out on albino mice of either sex weighing between 18-24 gm. 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  $23 \pm 2^\circ\text{C}$ .

### Material

Fresh Pineapples were purchased from local market in Karachi and were identified by Department of Pharmacognosy, Faculty of Pharmacy Jinnah University for Women. The Pineapples were then peeled, cut and blended and juice was filtered.

### Dosing Regime

The mice were equally divided into 3 groups (control, Standard and pineapple juice). The control mice were given distilled water 0.5 ml, Standard taken was Lorazepam 2 mg/60 kg that means 0.3 mg/kg, this dose was adjusted according to weight of mice in milligrams. Stock solution was prepared 12 mg/60 ml in distilled water and dose was administered by serial dilution method orally.

Pineapple juice was given 0.5 ml orally. All the groups were given dose once daily. The study was carried out for 15 days.

### Head Dip Test

Hole Board or Head Dip Test is used for identification of anxiety. The apparatus consists of an enclosed wooden rectangular box (35 cm×45 cm×45 cm). The holes are 2.5 cm in diameter and found in all walls [23].

### Procedure

Before beginning the experiment the mice were familiarized with the environment in which the Hole Board apparatus was placed. The temperature was maintained same as in animal house. The mice that were ignorant of the apparatus were placed in the centre area and allowed to move freely for 5 minutes. The number of times the mouse stuck out its snout was noted. After each reading to clear off smell of animal the apparatus was cleaned with 70% alcohol [24].

### Open Field Test

Open field test is used to assess emotional behavior in rodents. It can be used to measure exploratory activity, locomotors activity and gives idea of initial anxiety related behavior in rodents [25]. It consists of large square chamber with walls 42 cm high. The floor is marked into 25 squares each 15 cm in diameter. A 15×15 cm central square of equal size are also present in middle arena of open field.

#### **Procedure**

The mouse was held gently by the tail and placed in centre of arena in open field one at a time. The number of times the mice moved in central and peripheral squares was recorded for 5 minutes. After performing experiment the apparatus was cleaned using 70% alcohol to remove the body odour of subject.

#### **Light and Dark test**

It was designed by Crawley and Goodwin (1980). Rodents generally favor dark areas. This test was used to assess anxiety behavior in rodents.

The chamber is divided into two compartments by dark partition having small aperture (13 ×5 cm) located at bottom. One compartment is painted black it is the dark compartment it is covered by a lid. The other has transparent sides and is uncovered. It is brightly lit using a lamp.

#### **Procedure**

The mice are acclimatized with the surrounding where the apparatus is placed. Each mouse is transferred individually in the centre of the brightly illuminated compartment. Mice are allowed to freely explore the compartments moving from light to dark. Record the transitions made by the mouse in the dark compartment. This test is conducted for 5 minutes [26].

#### **Elevated Plus Maze**

Is an animal model of anxiety which is based on rodent's aversion to open spaces and preference to remain near vertical surfaces (thigmotaxis).

#### **Procedure**

The apparatus consists of plus maze shape with two open arms and two closed arms, elevated 40-70 cms from floor. The mice are familiarized with environment and placed in center of maze. Record the number of times the mouse enters the open arms. This test is done for 5 minutes [27].

## **RESULT**

By applying SPSS 19 all the data has been analyzed. 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.

## **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 in order to incorporate them properly in diet and overcome symptoms of disease condition. **Table 1** shows the effect of Pineapple juice on head dip activity.

**Table 1. Effect of Pineapple Juice on Head Dip**

| Groups           | Mean ± SD | P (Control) | P (Standard) |
|------------------|-----------|-------------|--------------|
| Control          | 47 ± 1.1  |             |              |
| Pine apple Juice | 18 ± 1.03 | 0.000       | 0.126        |
| Standard         | 17 ± 1.7  |             |              |

Initially when animal is placed in apparatus it tries to find an escape route due to fearful and neophobic response [28] further confirmation has been attained by literature search showing elevated level of corticosteroids when exposed to apparatus [29]. 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 [30]. This postulation supports our above results that Pineapple juice possesses anxiolytic effect. Flavonoids are the main components present in Pineapple juice responsible for most of the medicinal properties [31]. **Table 2** shows the effect of Pineapple Juice on Peripheral squares whereas **Table 3** shows effect on Central squares.

**Table 2. Effect of Pineapple Juice on Peripheral Squares**

| Groups           | Mean ± SD  | P (Control) | P (Standard) |
|------------------|------------|-------------|--------------|
| Control          | 173 ± 1.78 |             |              |
| Pine apple Juice | 66 ± 1.39  | 0.000       | 0.000        |
| Standard         | 49 ± 1.44  |             |              |

**Table 3. Effect of Pineapple Juice on Central Squares**

| Groups           | Mean ± SD | P (Control) | P (Standard) |
|------------------|-----------|-------------|--------------|
| Control          | 7 ± 1.03  |             |              |
| Pine apple Juice | 14 ± 1.05 | 0.000       | 0.000        |
| Standard         | 19 ± 0.7  |             |              |

Our results indicate that the numbers of peripheral squares were highly significantly reduced when compared to control whereas it was highly significantly greater than standard. Similarly the number of central square crossings were highly significantly increased as compared to control and decreased as compared to standard. The central square crossings indicate anxiety and exploratory behavior [32]. Increase frequency indicates high exploratory behavior and low anxiety. **Table 4** shows effect of Pineapple juice on light and dark model.

**Table 4 Effect of Pineapple Juice on Light and Dark Model**  
(Light Transition time)

| Groups           | Mean ± SD  | P (Control) | P (Standard) |
|------------------|------------|-------------|--------------|
| Control          | 44 ± 1.19  |             |              |
| Pine apple Juice | 136 ± 1.66 | 0.000       | 0.000        |
| Standard         | 234 ± 1.67 |             |              |

Our results indicate highly significant increase time spend in light box as compared to control whereas as compared to standard the time was highly significantly decreased. Research studies have shown that an animal who is stressed or in fear or has anxiety tends to stay for prolonged period in darker area. He will not prefer to move and explore about the white box and peaking between two boxes will also be low. On the other hand anxiolytic drugs increase the number of transitions and the time spend in the white area [33]. **Table 5** shows effect of Pineapple juice on elevated plus maze.

**Table 5: Effect of Pineapple Juice on Elevated Plus maze**  
(Time spend in open arm)

| Groups           | Mean ± SD | P (Control) | P (Standard) |
|------------------|-----------|-------------|--------------|
| Control          | 37 ± 0.67 |             |              |
| Pine apple Juice | 79 ± 2.3  | 0.000       | 0.000        |
| Standard         | 73 ± 3.7  |             |              |

Note: Values are mean ± S.D, N=10=number of animals, \*p<0.05=significant, \*\*\*p<0.0001=highly significant IS=insignificant difference.

Our results show highly significantly increased time spend in open arms as compared to control and standard. This shows that pineapple juice possesses anxiolytic effects which are highly significant when compared with control but highly significantly reduced when compared with standard drug.

It is a known fact that majority of anxiolytic drugs act by mediating effect on GABA neurotransmitter by binding to GABA<sub>A</sub> 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 [34]. Phenolic compounds are important constituent of Pineapple and it might possess anxiolytic effect by Gabanergic mechanism. Besides this the antioxidant effect produced by flavonoid component is also thought to provide neuroprotective effect in mice hippocampus [35].

The standard drug taken was lorazepam an intermediate acting benzodiazepine which possesses anxiolytic effect at low doses and hypnotic effect at high doses [36]. The mechanism of action is by potentiating gabanergic effect. Our results show similar but reduced effects as compared to lorazepam. During the period of study all the animals survived and no side effect were observed because the low dose was administered.

From the above study we can conclude that Pineapple juice 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.

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