

## Evaluation of Neurobehavioral Effect of *Citrullus Lanatus* Juice on Albino Mice

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### ABSTRACT

**Objective:** To observe the effect of *Citrullus lanatus* var. *lanatus* on albino mice model of anxiety and depression.

**Methodology:** This experimental study was done in the Pharmacology department of Karachi University for a period of 02 months from 5<sup>th</sup> February-9<sup>th</sup> April 2016. Watermelon (*Citrullus lanatus* var. *lanatus*) was used. Animals were randomly assigned into 4 groups A, B, C and D (n=10). Group A was control (normal saline). Group B was treated with *Citrullus lanatus* juice in a dose of 3 ml/kg, Group C was given 6 ml/kg of juice. Two drugs (Diazepam and Fluoxetine) were used as standard and given to group D animals. All doses were administered orally. Level of anxiety and depression among animals was tested by light/dark box test and forced swim test. Data was analyzed statistically.

**Results:** Animals treated with *Citrullus lanatus* juice showed marked reduction in anxiety which was evident from results of light/dark box test while forced swim test revealed no effect on depression.

**Conclusion:** *Citrullus lanatus* contains important natural anti-oxidants like lycopene and vitamin C which prevent injury to vital organs like liver, heart and brain from toxic effects of reactive oxygen species. Arginine in *Citrullus* juice is also precursor of neurotransmitters like  $\gamma$ -Aminobutyric acid (GABA) and glutamate. These results showed that *Citrullus* because of its components like arginine played an important role in maintaining normal neurological function and ameliorated anxiety and depression.

**Keywords:** Anti-anxiety effect, Anti-depressant effect, *Citrullus lanatus*, Diazepam, Fluoxetine.

### INTRODUCTION:

Medicinal plants have a significant role in pharmacological research as well as for drug developers. Presently beneficial constituents of plants are used as therapeutic agents directly, as well as utilized by pharmaceutical companies to form active compounds of drugs. More and more plants must be preserved to ensure their availability in future as it is the only way by which essential plants can be conserved<sup>1</sup>.

Plants play a very important role in prevention and treatment of diseases due to their medicinal effects, and are being used globally for this purpose<sup>2,3</sup>. Fresh fruits and vegetables are very important food components and they cannot be removed from diet. It is the ability of human body which can adapt itself physiologically for different types of food<sup>4</sup>. Fruits and vegetables yield less amount of energy, that's why they are beneficial for weight management, but they are also sufficient source of fibres, vitamins and minerals<sup>5</sup>.

Egusi watermelon, West African watermelon, Egusi melon, Desert watermelon, and cooking melon are different names of *Citrullus lanatus*<sup>6</sup>. It belongs to the family Cucurbitaceae<sup>7</sup>. Total amount of energy derived from 100gm of watermelon is 30 kcal. 92% content is

water, 7.55% consist of carbohydrates which can be further divided into sugar (6.2%) and dietary fiber (0.4%). It contains low calories because it is cholesterol and fat free<sup>8,9</sup>. *Citrullus lanatus* juice also contains important amino acids, 71% of which include Arginine and Citrulline<sup>10</sup>. *Citrullus lanatus* juice also contains many beneficial components such as flavonoids, alkaloids, glycosides, polyphenols, steroids and tannins which produce beneficial effects on body<sup>11</sup>.

Arginine, an amino acid, is an essential component of *Citrullus lanatus* juice. Three important end products are usually derived from catabolism of arginine, each of which acts as cell signaling mediator and includes nitrous oxide (NO), Glutamate and Agmatine. GABA mainly regulates ion channel and is derived from glutamate<sup>12</sup>.

Anti-anxiety drugs work by elevating GABAergic neurotransmission in brain which is the mode of action of most anxiolytic drugs<sup>13</sup>. One-eighth population worldwide is affected from anxiety, that's why in the research field of psychopharmacology, it has gained significant attention<sup>14,15</sup>. There are many drugs used for treatment of stress and anxiety but they have adverse effects too<sup>16</sup>.

It is also believed that GABA and cortical GABA-A receptors are deficient in depressive patients. So, drugs which elevate or stimulate GABAergic system have important impact on depression<sup>17</sup>. It is evident from some studies that deficiency of GABAergic transmission can result in depression<sup>18</sup>. However, little work has been done in this regard. With this background, this study was undertaken to observe the effects of *Citrullus lanatus* on anxiety and depression in experimental animals.

### METHODOLOGY:

This experimental study was conducted in the research institute of pharmacology department,

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University of Karachi from 5<sup>th</sup> February 2016 to 9<sup>th</sup> April 2016 for 2 months. Watermelon (*Citrullus lanatus*) was purchased from the local fruit market. Sample was taken when it was available in fresh state for experimental analysis. Fresh watermelon juice was prepared on daily basis with the help of muslin cloth.

Mice having weight ranging from 20 to 25gram were used for this study. They were kept under standard laboratory conditions. Free access to water and food was permitted. They were kept in wired gauzed plastic cages in animal house in the Pharmacology department of Karachi University. They were acclimatized for 1 week before the start of administration of *Citrullus lanatus* juice orally, and kept on standard mice pellet. Animals were randomly assigned in groups A, B, C and D, with 10 animals in each group.

Group A was marked as control and was given normal saline. Group B was treated with 3 ml/kg of *Citrullus lanatus* juice (CLJ 3 ml/kg), Group C was treated with 6 ml/kg *Citrullus* juice (CLJ 6 ml/kg)<sup>19</sup>. Two drugs were used as standard and were administered to group D. All doses were administered orally. Group D was further divided into two subgroups; D1= Diazepam 1mg/kg<sup>20</sup> and D2=Fluoxetine 20 mg/kg<sup>21</sup>. Neurobehavioral studies were carried out on 7<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day by light/dark box test and forced swim test. The results of the treated groups were compared with control group after taking mean of all values. Significance of mean was determined by Tukey's post-hoc test. P-value less

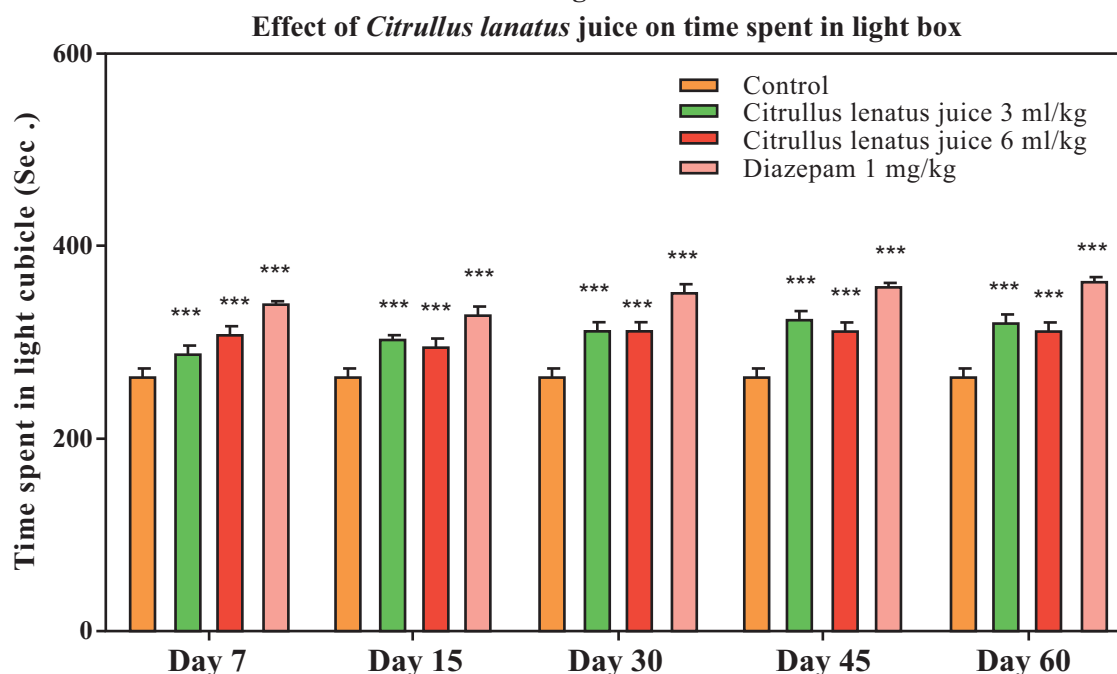
than 0.05 was considered as significant.

## RESULTS:

**Light/Dark Box Test:** A significant contrast was seen in light/dark box test between four gatherings of mice, in time spent in light box area by one-way ANOVA ( $F_{3, 16}=51.32, P < 0.001$ ) and time consumed in dark cubicle ( $F_{3, 16}=31.42, P < 0.001$ ) where F was the variation between the groups. Tukey's post-hoc test determined that time spent in light cubicle area was significantly increased in treated groups (CLJ 3 ml/kg and CLJ 6 ml/kg) when compared with the control group (Figure-1a). Whereas, time spent in the dark cubicle was significantly reduced when compared with the saline-treated control group (Figure-1b). Animals after 07, 15, 30, 45 and 60 days of *Citrullus lanatus* treatment showed significant time consumed in light cubicle and reduced in dark cubicle.

**Forced swim test:** A significant difference was observed among four groups of mice in immobility time, i.e., time during which mice did not show any struggle ( $F_{3, 12}=782.8, P < 0.001$ ) in forced swim test, by one-way ANOVA, where F was the variation between the groups. Tukey's post-hoc test indicated that there was insignificant effect on immobility time in treated groups B and C when compared with the saline-treated animals (Figure-2). However, difference was significant among the treated groups and the standard drug group.

Figure-1a

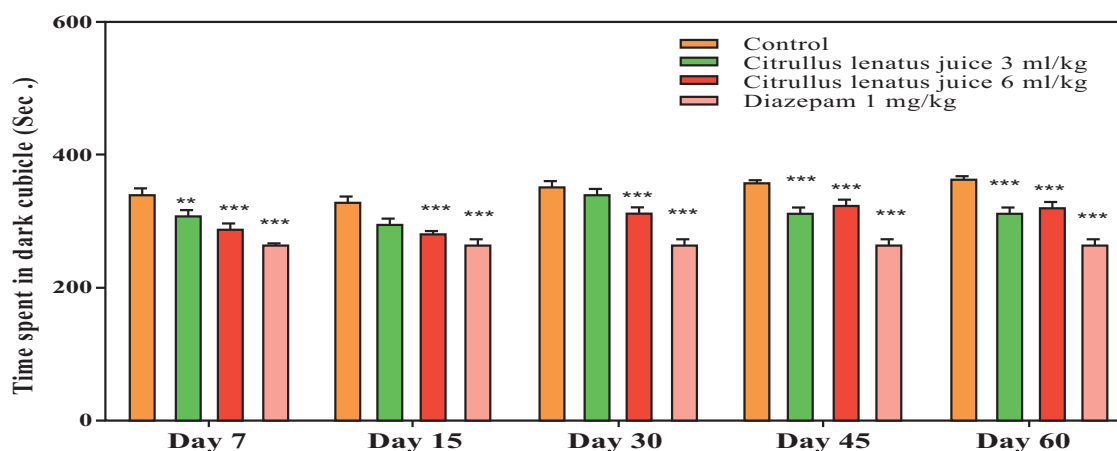


Number of mice per group (n) = 10.

Perceptions written as mean  $\pm$  S.E.M

\*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05; ANOVA took after by Tukey's test.

**Figure-1b**  
Effect of *Citrullus lanatus* juice on time spend in dark box

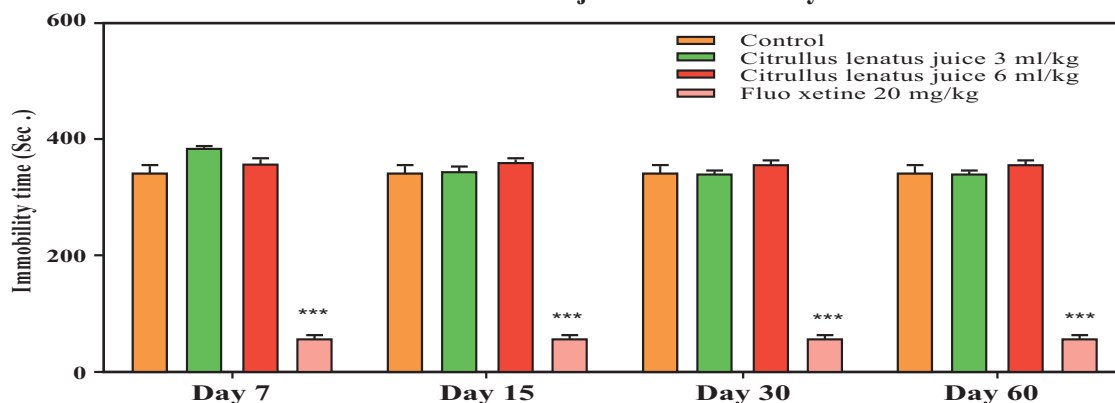


Number of mice per group (n) = 10.

Perceptions written as mean ± Standard error of mean.

\*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05; ANOVA took after by Tukey's test.

**Figure-2**  
Effect of *Citrullus lanatus* juice on immobility time



Number of mice per group(n) = 10.

Perceptions written as mean ± Standard error of mean.

\*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05; ANOVA took after by Tukey's test.

**DISCUSSION:**

All medicines have adverse effects, although they can be mild. Even newly discovered drugs have some side effects despite being expensive. Medicines obtained from plants are efficient and less expensive with less side effects<sup>22</sup>.

*Citrullus lanatus* has been used as a herbal medicine for some diseases such as hypertension, erectile dysfunction, jaundice and hepatomegaly<sup>23</sup>.

Light/dark box test was used to evaluate anxiolytic effect of *Citrullus lanatus* as compared to control and standard drug groups. It was evident from results that *Citrullus*

*lanatus* juice treated groups showed significant time spent in light box as compared to control group, whereas time spent in dark box reduced markedly. It was thus clear that *Citrullus lanatus* juice possessed anxiolytic activity. As mentioned earlier Arginine is present in its juice in sufficient amounts, which is precursor of GABA neurotransmitter and raised levels of GABA in brain have anxiolytic effect. Anxiolytic drugs act by increasing GABA activity or GABAergic neurotransmission in brain as reported<sup>13</sup>. Arginine-derived raised GABA levels might be the reason of anxiolytic effect as long term use of *Citrullus* juice significantly increased the time

utilized in light box and reduced in dull box.

Depression in animal models can be well evaluated by Forced swim test. Reduced amount of GABA and GABA- A receptors in brain are connected with depression and drugs which resemble GABA nergic system have effects on depression as mentioned above<sup>17</sup>. It is also evident from some studies that GABAnergic deficiency can lead to depressive state as already mentioned above<sup>18</sup>, Tannins and flavonoids present in *Citrullus lanatus* juice can lead to depressive state<sup>24, 25</sup>. Results obtained from forced swimming test revealed that long term use of *Citrullus lanatus* juice had no significant impact on depression, as it might be the result of tannins and flavonoids present in its juice, which could cause depression in CNS neutralizing the anti-depression action of GABA, thus no significant effect occurred in depression in treated groups when compared with control.

### CONCLUSION:

It is concluded that *Citrullus lanatus* juice had excellent anxiolytic effect which was demonstrated by results of light/dark box test, but there was no significant effect on depression.

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