WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 211-219 Research Article ISSN 2278 – 4357

MEMORY BOOSTING EFFECT OF CITRUS JUICES

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Article Received on
15 July 2018,

Revised on 05 August 2018,
Accepted on 25 August 2018

DOI: 10.20959/wjpps20189-12313

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ABSTRACT

Introduction: Memory is very much influenced by elements such as, quality of sleep, Stress and food. There is an emergent proof to imply that citrus fruits with flavonoids and foods rich in flavonoids might exhibit reformative activity in such neuronal and memory enhancement, thereby impede the progression of disease condition. *Citrus sinensis* (sweet orange) and *Citrus paradisi* is widely known for its health-related benefits though it has been observed the growing use of dietary and herbal compounds are employed as treatment of anxiety and depression. **Objective:** The objective of this study was to describe the memory and learning properties of Citrus juices. **Study Design:** To evaluate the memory boosting effect of Citrus juices, Citrus sinensis and Citrus paradisi juice were used at three different doses alone and their two combinations in adult albino mice using Passive Avoidance response apparatus named Harvard Panlab controlled through LE2708 Programmer. **Settings:** To assess short or long-term memory of small animals Passive avoidance is used that is fear-motivated tests. **Method:** To assess the working memory of mice through passive avoidance by measuring latency to enter into the black compartment in two different sessions, that is, conditioning phase and Non-conditioning or test phase. **Results:** Animals showed highly significant increase at HCSD after 3 hr as compare to control latency time. There was highly significant increase in test latency time at MCPD observed after 3 hr and significant increase after 24 hr as compare to control latency time. There was significant increase at SPJ-1 and SPJ-2 observed after 3 hr in comparison with control latency time. **Conclusion:** It can be concluded that intake of fruit flavonoids results in

Cognitive changes and most likely it involves morphological changes triggered by the direct actions of flavonoids on neuronal signalling.

KEYWORDS: Learning, *Citrus sinensis* (sweet orange), *Citrus paradisi.*

INTRODUCTION

Passive avoidance test is performed to assist long term and short term memory on laboratory animals. This test is a fear motivated test. Several researches have showed that synaptic changes affect learning and memory. Since post-synaptic NMDA receptors depolarization and pre-synaptic glutamate release leads to calcium ion entrance that triggers intracellular signaling cascades, such as gene translation and transcription. cAMP, CREB and PKA pathways play important role in NMDA receptor signaling. In cellular level calcium influx increases the making of cAMP by adenylyl cyclases, which is linked to G-proteins and act as non-glutamate transmitters. cAMP leads to CREB activation due to the activation of PKA (Cho et al., 2013). During memory formation extra cellular signal-regulated kinase (ERK) gives control over CREB- mediated transcription (Williams et al., 2012). CREB binding proteins (CBP) regulates the genes which are made up of protein components, required in stabilization and development of synapses required for memory. Recently it is observed that CBP and CREB by histone acetylation cause long term epigenetic changes in chromatin structure and mediate memory (Wilcox et al., 1998; 1999; Shang et al., 2005; Hernandez et al., 2008).

Plant-based phytochemical has been found to be associated with neural performance and memory, such as flavonoids, which may help in regeneration of neurons and shows protective effect against different types of neuronal death (Spencer, 2010). Phytochemicals may be extracted from fruit juices (Deyhim et al., 2006; Xu. 2008), but duration of extraction and storage conditions may affect bioavailability of these phytochemicals. Therefore to avoid this loss we used freshly squeezed juices of Citrus sinensis (sweet orange) and Citrus paradisi (Grapefruit).

Due to poor evidence of scientific and clinical data to demonstrate their effectiveness and safety is a main obstacle in amalgamation of plant based medicine in contemporary medical practices. In herbal drugs developing and manufacturing simple bioassays for biological consistency, pharmacological and toxicological assessment there is a need to perform clinical

research, and developing diverse animal models for toxicity and safety assessment. It is also extremely essential to formulate the active components from these plant extracts.

OBJECT OF STUDY

The objective of present study was to describe the memory and learning properties of Citrus sinensis, Citrus paradise and their combination juices, to evaluate the fruit-derived phytochemicals, in particular flavonoids, are capable of promoting beneficial effects on memory and learning.

MATERIAL AND METHOD

Animals: Current study was performed after the approval of the protocol by Board of Advance Studies and Research, University of Karachi. Adult albino mice with an average body weight of 25±10 grams were kept under controlled environment of temperature 23±2°C and humidity 50-60%. All animals (N=90) were grouped into nine, with ten animals in each group. All animals were allowed to have free access to food and water. Animals of control group received sterile water through oral route for fifteen consecutive days, while other groups received citrus paradisi, citrus sinensis and combination of juices for same period of time between 8.00 am to 10.00 am on once daily basis through oral route.

Dosing

Citrus Sinensis Juice

C. sinensis (Sweet orange) were purchased from local market, identified by center of plant conserva-tion, University of Karachi and voucher specimen no CS-10-10 was deposited in Department of Phar-macognosy, University of Karachi. Fruit samples were peeled and squeezed by hand, fresh juice so yielded was utilized soon after filtration.

C. sinensis juice was orally administered in three doses that is, 2, 5 and 8ml/kg according to body weight and considered as low (LCSD), moderate (MCSD) and high (HCSD) doses.

Citrus Paradisi Juice

*C. Paradisi(Grapefruit)*were purchased from local market, identified by center of plant conservation, University of Karachi and voucher specimen no CP-09-10 was deposited in Department of Phar-macognosy, University of Karachi. Fruit samples were peeled and squeezed by hand, fresh juice so yielded was utilized soon after filtration.

C. Paradisi juice was orally administered in three doses that is, 0.1, 0.3and 0.5ml/kg according to body weight and was considered as low (LCPD), moderate (MCPD) and high (HCPD) doses.

Combinations of *C. sinensis* and *C. paradisi*

C. sinensis and *C. paradisi* juices were also given orally in two combination doses that is, 2 +0.1 and 5+0.3ml/kg and were abbreviated as SPJ-1 and SPJ-2 respectively.

In this experiment animals were used in accordance with the National Institute of Health (NIH) guide for the care and use of Laboratory Animals (National Research Council 1996) and approved by the Board of Advance Studies and Research of Karachi University.

Design of experiment

Passive Avoidance apparatus named Harvard Pan lab controlled by LE2708 Programmer was used to evaluate working memory of mice by measuring latency to enter into the black compartment. It is a fear-motivated trial used to evaluate short term and long-term memory of laboratory animals in two sessions, Condition session and Non-condition or test session. In condition session, entry of mice from white compartment separated by guillotine gate into the black compartment was punished for 2 seconds with 0.8 mA inescapable electric shock. Conditioning phase of one trial consist of 1 minute exploratory phase. After this cross latency was measured i.e. mice exit white compartment and enters into black and gate closed (Mice did not enter into black compartment before cut-off latency of 1 minute were eliminated from the experiment). Test phase of one trial was performed by retesting the mice after 3 and 24 hours to assess short term and long term memory. In these test mice were kept in white box with door opened and latency to enter into dark box was measured.

Statistical analysis

Data entry and analysis was performed using 17th version of Superior Performance Statistical Software (SPSS). Data was presented as mean ± S. E. M with 95% confidence interval. Analysis of variance (ANOVA) followed by post hoc was performed for comparisons of values with control. Values of $p \leq 0.05$ were considered statistically significant and $p \leq 0.005$ as highly significant.

Passive Avoidance

Figure-1 Illustrates the impact on memory index by comparing the latency to go to the black compartment between the conditioning and the test phase in three doses of *C. sinensis* and control animals after 3 hr and 24 hr. It was observed significant increase in test latency time at MCSD after 3 hr and at HCSD after 24 hr. While highly significant increase was observed at HCSD after 3 hr in comparison to control latency time. On the other hand insignificant change was observed at LCSD after 3 hr, MCSD and LCSD after 24 hr as compare to control latency time.

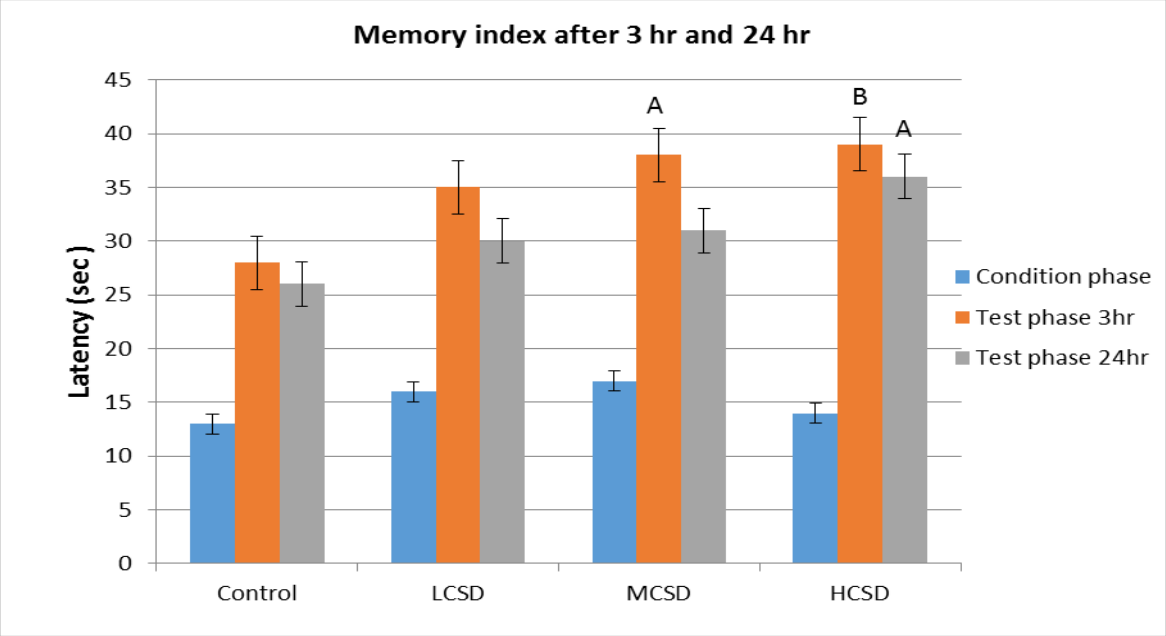

Fig. 1: Effect of Citrus sinensis on learning and memory.

n=10

Values are Mean ± S.E.M

P≤0.05 significantly different, Columns (A)

P≤0.005 highly significant, Column (B)

Figure-2 Illustrates memory index by comparing the latency to go to the black compartment between the conditioning and the test phase in three doses of *C. paradisi* and control animals after 3 hr and 24 hr. highly significant rise was observed in test latency time at MCPD after 3 hr and significant increase after 24 hr in comparison to control latency time. While there was significant increase observed in test latency time at HCPD after 3 hr in comparison with control latency time. On the other hand insignificant change was observed in test latency time at LCPD after 3 and 24 hr as compare to control latency time.

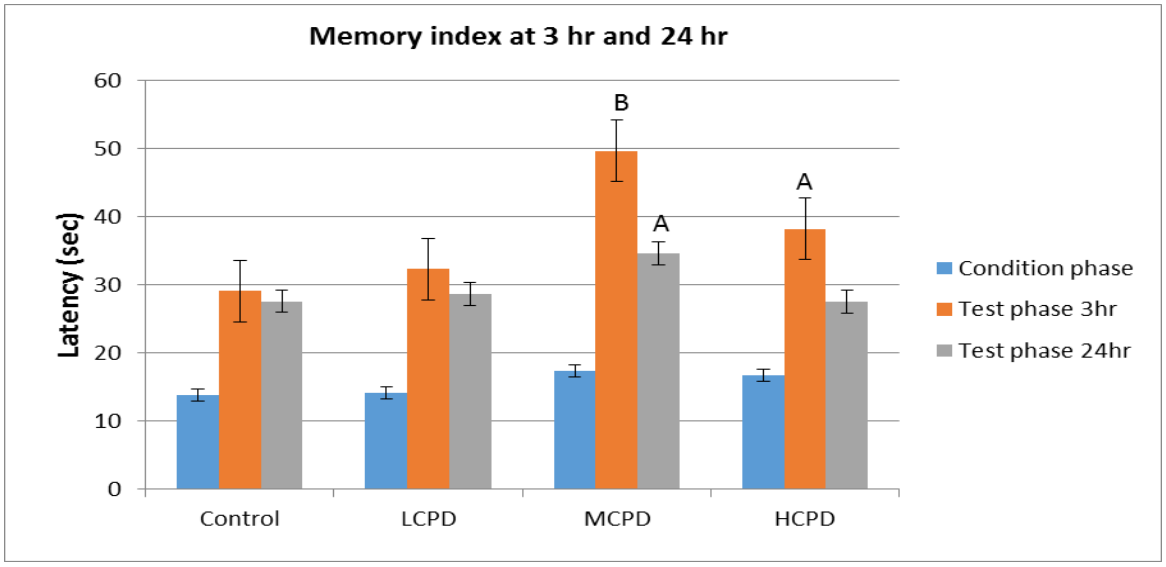

Fig. 2: Effect of *C. paradisi* on learning and memory.

n=10

Values are Mean ± S.E.M

P≤0.05 significantly different, Columns (A)

P≤0.005 highly significant, Column (B)

Figure-3 Illustrates memory index by comparing the latency to go to the black compartment between the conditioning and the test phase in two combined doses of *C. sinensis and C. paradisi* after 3 hr and 24 hr. There was significant increase at SPJ-1 and SPJ-2 observed after 3 hr in comparison with control latency time. While there was significant rise at SPJ-2 after 24 hr as compare to control latency time. Though an insignificant change was observed at SPJ-1 after 24 hr as compare to control latency time.

Fig. 3: Effect of combined doses of *C. sinensis* and *C. paradise* on learning and memory.

n=10

Values are expressed as Mean ± S.E.M

Columns (A) are significantly different, P≤0.05

Column (B) highly significant, P≤0.005

DISCUSSION

Flavonoids are naturally occurring molecules abundantly found in fruit and vegetables; number of studies has suggested an inverse association between flavonoids intake and various disorders. Is flavones, flavanols and anthocyanins are main flavonoids, which have shown positive effects on the brain cells, while Citrus sinensis and citrus paradisi are rich in all these flavonoids. Recent study rotates around the synaptic change affecting learning and memory, since flavonoids have direct role in signaling pathways modulating cAMP, CREB and PKC (Wilcox et al., 1999). Present study revealed significant increase in short and long term memory by orange juice in dose dependent manner and Citrus paradise showed highly significant result at moderate doses, there was significant increase at SPJ-1 and SPJ-2 observed after 3 hr in comparison with control latency time. While there was significant rise at SPJ-2 after 24 hr as compare to control latency time. The latency values of juices reveals that components present in Citrus paradisi and Citrus sinensis juices may be responsible for synergistic response. Previous studies suggest that administration of flavonoid as dietary supplement have useful effects on blood supply to the brain, hence improves learning and memory (Shang et al., 2005, Cambay et al., 2011). Flavanols and anthocyanins have beneficial effects on memory since interact with neuronal signaling pathways, mitogenactivated protein kinase (MAPK) and the phosphotidylinositol-3 kinase (PI3-kinase) both having important role in morphological process controlling memory storage in hippocampus and cortex (Williams and Spencer, 2012). Another possible mechanism through which flavonoids enhance memory is binding to regulatory proteins for example cAMP response element-binding protein (CREB), responsible for the expression of important genes linked to memory. Flavonoids may also increase neuronal protein synthesis that leads to further synapses and neurotransmitters formation increasing the power of communication in both flow of information and neurons (Spencer, 2010). Results of present study also reveal favorable effect on memory therefore it may be suggested that flavonoids abundantly present in Citrus paradisi and Citrus sinensis may be responsible for the memory boosting effect of these fruits alone as well as in combination. Another study suggests that flavonoids possibly support CREB activation and boost the levels of (BNF) brain-derived neurotrophic factor in

the hippocampus. Since CREB is necessary for the production of neutrophins-protein, that may lead to neuronal survival, differentiation and function (Hernandez and Abel, 2008). Recent report of Cho et al., (2013) suggests that antioxidant action of flavonoids affect memory and learning. They modulate antioxidant enzyme activity or interrupt signaling cascade and plays important role in blocking oxidative neuronal injury.

CONCLUSION

From the results of the present study it is concluded that with long-term use of fruit flavonoids the observed Cognitive changes are most likely due to morphological changes which involve the direct action of fruit flavonoids on neurons and their signalling potential However, presently it is unknown the degree to which regular heightened improvement in blood flow of brain which results in such changes. Thus, further studies are required and should be intended to evaluate the exact chronological nature of flavonoids which effects on memory along with other issues, for example when one needs to start using flavonoids to achieve the maximum beneficial results.

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