Memory boosting effect of Citrus limon, Pomegranate and their combinations

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Abstract: Memory is greatly influenced by factors like food, stress and quality of sleep, hence present study was designed to evaluate the effect of Citrus limon and Pomegranate juices on memory of mice using Harvard Panlab Passive Avoidance response apparatus controlled through LE2708 Programmer. Passive avoidance is fear-motivated tests used to assess short or long-term memory of small animals, which measures latency to enter into the black compartment. Animals at MCLD showed highly significant and significant increase in latency to enter into the black compartment after 3 and 24 hours respectively than control, animals at HCLD showed significant increase in latency only after 3hours. Animals both at low and moderate doses of pomegranate showed significant increase in test latency after 3 hours, while animals at high dose showed highly significant and significant increase in latency after 3 and 24 hours respectively. There was highly significant and significant increase in latency in animals at CPJ-1combination after 3 and 24 hours respectively; however animals received CPJ-2 combination showed significant increase in latency only after 3 hours as compare to control. These results suggest that Citrus limon and Pomegranate has phytochemicals and essential nutrients which boost memory, particularly short term memory. Hence it may be concluded that flavonoids in these juices may be responsible for memory enhancing effects and a synergistic effect is observed by CPJ-1 and CPJ-2 combinations.

Keywords: Learning, Citrus limon, pomegranate.

INTRODUCTION

Inherently mouse prefers to travel into dark, when placed in the light box, but when punished escape to enter into dark which is measured as a parameter of working memory, hence this test evaluates the learning ability and memory. Thus passive avoidance is fear-motivated tests typically used to assess shorter long-term memory of small laboratory animals by measuring latency to enter into the black compartment after punished by a mild inescapable electrical shock during conditioning session.

Passive avoidance learning is based on contextual memory (memory related to place and events) and amygdale dependent emotional memory (memory related to fear). Hippocampus plays an important role in contextual memory, hence performance of Passive avoidance learning decreases by defect of either contextual or emotional memory.

Present study revolves around the synaptic change that could affect learning and memory. Since pre-synaptic glutamate release and depolarization of post-synaptic NMDA receptors leads to entry of calcium and triggers a wide range of intracellular signaling cascades, such as plasticity-linked gene transcription and translation. Activation of cAMP response element-binding protein (CREB) plays an important role in memory by regulating variety of plasticity-linked gene. Its activation occurs in two ways; cAMP induced protein kinase activator (PKA) (Cho *et al.,* 2013) and extra cellular signal-regulated kinase (ERK) pathway with phosphotidylinositol-3kinase (PI3-kinase) and protein kinase C. CREB regulated genes are protein in nature required in development and stabilization of synapse needed in memory function (Shang *et al.,* 2005; Hernandez & Abel, 2008; Williams & Spencer, 2012).

Mitogenic-activated protein kinase (MAPK), phospholipase C (PLC) and phosphotidylinositol-3 (PI3) kinase are other signal transduction enzymes which play important neuronal role in morphological mechanism controlling storage of memoryin the hippocampus and cortex (Williams & Spencer, 2012).

Role of flavonoids have been found to be associated with memory and neural performance. They may aid in regeneration of neurons and provide protection against different types of neuronal death (Spencer, 2010).

Previous studies suggest that is flavones, flavanols and anthocyanins are main classes of flavonoids, which shows positive effects on the brain cells. Citrus limon and Pomegranate are rich in these phytochemicals (Wilcox *et al.,* 1999; Fischer *et al.,* 2011). However duration of extraction and storage conditions of juices could affect

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bioavailability of these phytochemicals (Deyhim *et al.*, 2006; Xu *et al*., 2008). Thus to avoid loss of ingredients present study was carried out on fresh juices of Citrus limon and Pomegranate fruits (Riaz *et al.*, 2013).

MATERIALS AND METHODS

Animals

Present study was conducted after the approval of the protocol by Board of Advance Studies and Research, University of Karachi. Adult albino mice with a mean body weight of 25±10 grams were kept under controlled condition of temperature 23±2°C and humidity 50-60%. All animals (N=90) were divided into nine groups, with ten animals in each group. All animals had free access to food and water ad libitum.

Animals of control group received sterile water through oral route for fifteen consecutive days, while other groups received citrus limon, pomegranate and combination of juices for same period of time between 8.00 am to 10.00 am on once daily basis through oral route.

Citrus limon juice

Citrus limon was purchased from local market, identified by center of plant conservation, University of Karachi and voucher specimen no C.L 11-11 was deposited in the Department of Pharmacognosy, University of Karachi. Citrus limon fruits were squeezed by hand to yield fresh juice, which was immediately filtered and administered by mouth in three doses low (LCLD), moderate (MCLD) and high (HCLD) according to body weight i.e. 0.2ml/kg, 0.4ml/kg and 0.6ml/kgrespectively (Riaz *et al.*, 2013).

Pomegranate juice

Pomegranate was also purchased from local market and identified by center of plant conservation, University of Karachi. The voucher specimen no P.G 11-12 was deposited in the Department of Pharmacognosy, University of Karachi. Fruits were peeled and squeezed to yield fresh juice which was immediately filtered and administered by mouth in three doses low (LPD), moderate (MPD) and high (HPD) according to body weight i.e. 2ml/kg, 5ml/kg and 8ml/kg respectively (Riaz *et al.*, 2013)

Combinations of Citrus limon and pomegranate

Citrus limon and Pomegranate juices were also given orally in two combination doses i.e. 0.4ml/kg Citrus limon +5ml/kg Pomegranate and was designated as CPJ-1, while another group designated as CPJ-2 received 0.2ml/kg Citrus limon +8ml/kg Pomegranate (Riaz *et al.*, 2013).

Design of experiment

Harvard Pan lab Passive Avoidance apparatus controlled through LE2708 Programmer was used to assess working memory of mice by measuring latency to enter into the black compartment. It is a fear-motivated test used to assess short term and long-term memory of small 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 placed in white compartment with door opened and latency to enter into dark compartment was measured.

STATISTICAL ANALYSIS

Data entry and analysis was performed using Superior Performance Statistical Software (SPSS) version 20. ANOVA followed by post hoc was performed for comparisons of values with control. Data was presented as mean ±S.E.M with 95% confidence interval, values of $p \leq 0.05$ were considered statistically significant and $p \leq 0.005$ highly significant.

RESULTS

Fig. 1 shows comparison of memory index i.e. latency to enter into the black compartment during the conditioning and test phase at three doses of Citrus limon and control animals after 3 and 24 hours.

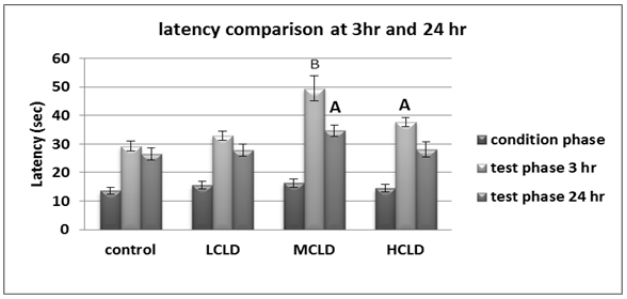

Fig. 1: Effect of Citrus limon on learning and memory.
n=10
Values are expressed as Mean ± S.E.M
Columns (A) are significantly different, P≤0.05
Column (B) difference highly significant, P≤0.005

There was highly significant increase in test latency at MCLD after 3 hours and significant increase after 24 hours as compare to control. While animals at HCLD showed significant increase in latency at 3hours than to

control. However no significant change in latency time was observed at LCLD both at 3 and 24 hours.

Fig. 2 shows comparison of memory index i.e. latency to enter into the black compartment during the conditioning and test phase at three doses of Pomegranate and control animals after 3 and 24 hours.

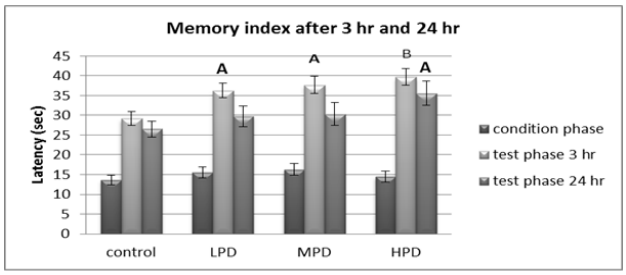

Fig. 2: Effect of Pomegranate on learning and memory
n=10
Values are expressed as Mean ± S.E.M
Columns (A) are significantly different, P≤0.05
Column (B) highly significant, P≤0.005

There was significant increase in test latency at LPD, MPD and highly significant increase at HPD after 3 hours than control. While animals at HPD showed significant increase in latency time after 24 hours than control. However no significant change in latency was observed both at LPD and MPD after 24 hours as compare to control.

Fig. 3 shows comparison of memory index i.e. latency to enter into the black compartment during the conditioning and test phase at two combination doses of Citrus limon plus Pomegranate and control animals after 3and 24 hours.

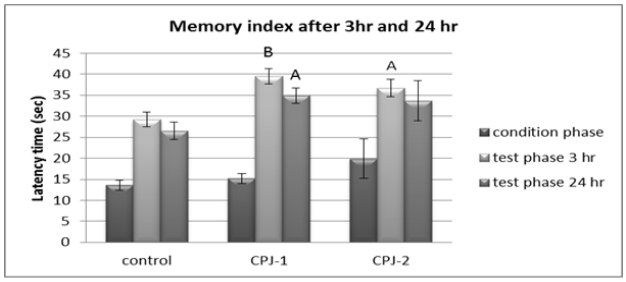

Fig. 3: Effect of combination doses of Citrus limon and Pomegranate on learning and memory
n=10
Values are expressed as Mean ± S.E.M
Columns (A) are significantly different, P≤0.05
Column (B) highly significant, P≤0.005

There was highly significant increase in latency time at CPJ-1 and significant increase at CPJ-2 after 3 hours as compare to control, but CPJ-1 group showed significant increase in latency only after 24 hours as compare to control.

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 limon and Pomegranate 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 Pomegranate in dose dependent manner and Citrus limon at moderate and high doses, while CPJ-1 combination showed highly significant increase in short term and significant increase in long term memory as compare to control whereas CPJ-2combination, showed only significant increase in short term memory. The latency values of CPJ-1 reveals that components present in Citrus limon and Pomegranate juices may be responsible for synergistic response.

Previous studies suggest that administration of flavonoid as dietary supplement have beneficial effects on blood flow 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, mitogen-activated protein kinase (MAPK) and the phosphotidylinositol-3 kinase (PI3-kinase) both having important role in morphological mechanisms controlling memory storage in hippocampus and cortex (Williams and Spencer, 2012).

Another possible mechanism through which flavonoids enhance memory is binding to regulatory proteins e.g. 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 more synapses and neurotransmitters formation increasing the strength of communication between neurons and flow of information (Spencer, 2010).

Results of present study also reveal favorable effect on memory therefore it may be suggested that flavonoids abundantly present in Citrus limon and Pomegranate may be responsible for the memory boosting effect of these fruits alone as well as in combination.

Another study suggests that flavonoids possibly promote CREB activation and increase the levels of 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

In conclusion it may be said that Citrus limon in moderate dose, Pomegranate in dose dependent manner and CPJ-1 combination of citrus limon and pomegranate are most effective in improving learning and memory.

These effects of fruit juices and their combination might be due to enhanced expression of genes linked to memory or increased neuronal protein synthesis that may lead to more neurotransmitter synthesis strengthening the communication between neurons.

However further studies on biochemical analysis, combination doses and large number of animals is required to reveal the exact role of these fruits on memory boosting.

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