

Chronic supplementation of *Juglan regia* improves antioxidant system in animal models of amnesia induced by Buscopan

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Abstract

Amnesia is characterized by an inability of a person that can no longer memorize or recall information that is stored in memory. Despite the fact that effective therapies for age-related cognitive decline and neurodegenerative illness are urgently needed. Walnuts (*Juglan regia*) seem to be abundant in phyto-chemicals, notably poly-unsaturated fatty acids, and may provide beneficial wellbeing effects, particularly to the brain. An alkaloid hyocine; having an anticholinergic activity which also suppresses the muscarinic responses of acetylcholine on post-ganglionic para-sympathetic neuro effector regions in the central nervous system, smooth muscle, and some secretory glands. Two different test group units and one control group of animals were created at random. One test group treated with buscopan while the

second test group administered buscopan+walnuts. Cognitive functions were evaluated using the Morris-water-maze while brain acetylcholine levels were also estimated. Antioxidant properties were evaluated by estimating malondialdehyde levels in brain. Present study showed that administration of buscopan impaired memory functions observed in increase in latency time and decrease in brain acetylcholine levels while these effects were significantly attenuated by administration of walnuts. Increased levels of malondialdehyde due to buscopan also decreased significantly with coadministration of walnut in brain.

Key Words: Amnesia, Walnut, Memory, Acetylcholine, Malondialdehyde

1. INTRODUCTION:

Hyoscine, which is derived from the Duboisia tree's leaves, has a derivative product named hyocin-butylbromide (HBB). Buscopan, or hyoscinebutylbromide, is a widely used antispasmodic chemical utilized because of its anticholinergic characteristics and also muscle

relaxing affects (Krueger et al 2013) [1]. Buscopan has the properties of quick action, improved effectiveness, and tolerance (Tytgat 2007).

Because of their high contents of proteins and polyunsaturated fatty acids and, walnuts

are considered to be nutritionally wealthy meal (Pribis and Shukitt 2014). The possible benefits of nuts on memory functioning, particularly among experienced individuals (Rosenegger 2010). Polyphenols have drawn the attention of scientists and food makers due to their possible significance in the prevention of different illnesses related to oxidative stress and its richness in naturally occurring foods (Scalbert et al 2005). Scientists suggested that dietary nutrients' significance in the preventing the conditions of oxidative stress-related illnesses such as neurodegenerative and heart disorders (Everitt et al 2006). Many researchers believe that consuming nuts may boost brain function because nuts can aid in depression and age-associated loss of brain functions (Ros et al 2021). Muthaiyah et al (2014) proposed that animal studies showed that walnuts could increase functioning of brain. They evaluate the walnuts effect Alzheimer's disease mice by improving their memory and learning skills significantly. Current research was planned to investigate the effectiveness of walnut as a food supplement on memory function in buscopan-induced amnesia in male rats.

2. MATERIAL AND METHODS

18 albino Wister locally bred (150-200 g) rats, bought from animal house of AKU (Aga Khan University Hospital). After bringing they were caged separately under a 12-hour light and dark cycle inside a silent place with maintained surrounding temperature ($22^{\circ}\text{C} \pm 2$). Animals

get easy approach to water and standard rodent diet. Initially animals were habituate for 3 days before experimentation. The rats were randomly divided into control groups (0.9 saline administered) and two test groups. Group 1 test animals were administered with Buscopan (5mg/kg/mL) for 20 days while group 2 test animals were given Buscopan+Walnuts (5mg+1g/kg/mL). The treatment methods were carried out in accordance with the National Institute of Health Guide for Laboratory Animal Care and Use (Publication No. 85-23, revised 2011). All experiment work was approved by the institutional ethical committee.

2.1 BEHAVIORAL METHODS

Working memory functionality in animals were investigated through Morris-water-maze apparatus. The test's equipment consisted of a circular tank which is of transparent glass. Liquid in the tank is opaque with a hidden platform. Experimentation by Morris water maze test is according to protocol in (Batool et al 2016).

2.2 NEUROCHEMICAL ESTIMATION

The Hestrin technique was used to determine the Acetylcholine (ACh) levels of the tissue as discussed in (Batool et al 2016). The enzyme was inactivated by boiling the tissue sample, which also released the associated ACh that reacted to ferric chloride. The brown colour that resulted from this reaction then measured at 540 nm in comparison to blank. In terms of mol/g of brain-tissues, ACh level was represented.

According to Procedure describe in (Haider et al 2015b) brain Malondialdehyde (MDA) concentrations were estimated as lipid peroxidation parameter which was measured as $\mu\text{mole/g}$ of brain-tissues

2.4 STATISTICAL ANALYSIS

Results are given as means \pm SD. Analysis of the data was performed through 1-way ANOVA using SPSS 20 version. Following Tukey's test was used for post hoc comparisons among groups. Value of $p < 0.05$ was considered as significant.

3. RESULTS

3.1 Memory Functions

Figure 1 expressed effects of administration of Buscopan and walnut on impairment of memory in rats. Data analysed by 1-way ANOVA showed; memory impairment effected significantly ($F=21.297, df=17, p < 0.01$). Oral

administration of Buscopan induced memory impairment that is improved by walnut.

3.2 Brain Acetylcholine levels

The effect of Buscopan and walnut administration on brain acetylcholine levels in rats expressed in figure 2. Data analysis through 1-way ANOVA expressed; memory impairment significantly effected ($F=51.332, df=17, p < 0.01$) indicated by decrease acetylcholine levels. But oral administration of walnut enhances brain acetylcholine level attributed to improvement of memory functions in rats.

3.3 Antioxidant effects

Antioxidant properties showed in figure 3. Analysis of data through 1-way ANOVA reveals that buscopan administration significantly ($F=26.14, df=17, p < 0.01$) increases MDA levels that were attenuated by walnut showing protective effects of nuts.

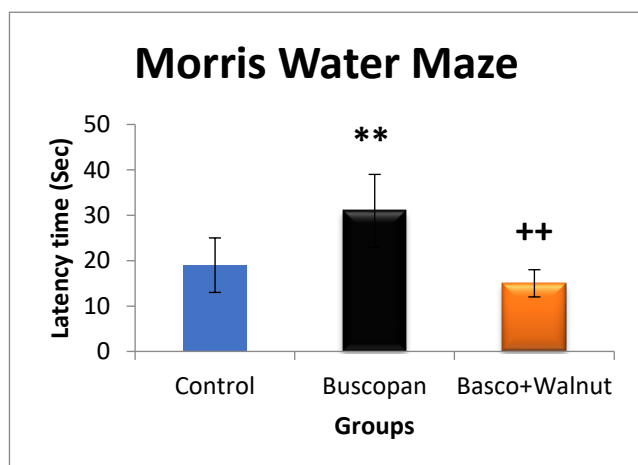


Figure 1: Values are mean \pm SD (n=6) significantly different through Tukey's-test analysed by 1-way ANOVA. ** $P < 0.01$ vs control, ++ $P < 0.01$ vs Buscopan administered rats.

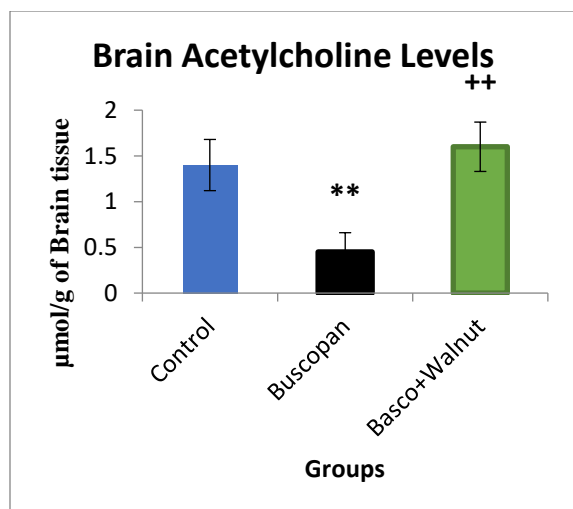


Figure 2: Values are mean \pm SD (n=6) significantly different through Tukey's-test analysed by 1-way ANOVA. **P<0.01vs control, ++P<0.01 vs Buscopan administered rats.

4. DISCUSSION

Administration of buscopan causes decrease in malondialdehyde levels representing decrease lipid peroxidase activity while walnut enhances antioxidant activity by increasing malondialdehyde levels in brain. In our current experiment buscopan administered for 20 days significantly impairs memory functions as indicated in Morris water maze test. It has been shown that hyoscinebutylbromide impairs cognitive function, particularly spatial acquisition and learning (Saraf et al 2011) in addition to this caused animals to become amnesic and decreases cholinergic function (Attrey et al 2012). In addition to being a memory impairment problem, amnesia is defined as a multidimensional ailment with a

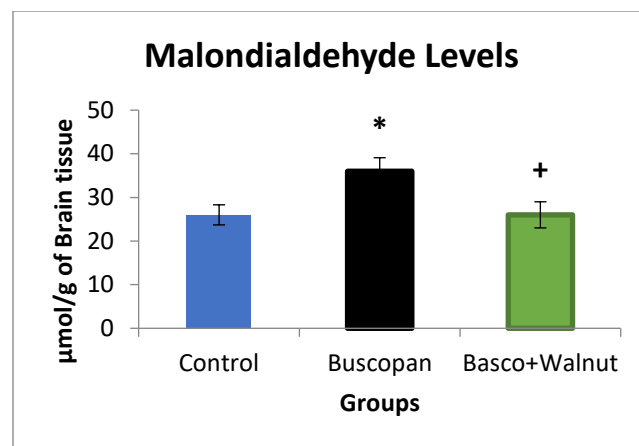


Figure 3: Values are mean \pm SD (n=6) significantly different through Tukey's-test analysed by 1-way ANOVA. *P<0.01vs control, +P<0.01 vs Buscopan administered rats.

typically dismal prognosis that results from more than 15 distinct forms of brain traumas and illnesses, including neurological and neurodegeneration disorders (Markowitsch 2012). For some scientist, amnesia has been acknowledged as a memory condition that can exist independently and as a result of memory loss or weakening (Karen and Langer 2019). Current study also shows that buscopan administered rats show increase in latency time indicated in Morris water maze test. This behavioral effect can be also attributed with the decrease brain acetylcholine levels. It has been studied that buscopan can potentially block nicotinic acetylcholine receptors (Weiser and Just 2009) that could leads to memory impairment.

Scientists reported that numerous phytochemicals are abundantly found in walnuts

such as omega-3 fatty-acids, which possess beneficial effects on brain function. As the brain ages, oxidative stress and chronic inflammation are major factors that can be reduced by its phenolic content. Health benefits of walnuts for the brain, muscles, and cognition showed significant improvements. Various cognitive domains, like learning and memory. Another benefit of using walnut is that the weights of the rats remain constant before and after implication. According to a previous study, some dietary habits and minerals may improve brain health and lower the risk of dementia through improving brain function (Whalley et al 2004). Walnut has been found to aid memory and learning in animals with an Alzheimer's disease-like condition when consumed as a dietary supplement (Muthaiyah et al 2014). The Mediterranean diet usually includes a lot of nuts in its meals (Willett et al 1995). Previous researches on both human and animal studies showed that nuts may have a great influence on memory functioning Pribis and Shukitt-Hale 2014). Previously assessed improved memory retention following nuts consumption has been attributed to their high unsaturated fatty acid content (Willis et al 2009a). The current research showed that oral walnuts administration following buscopan administration indicates improvement in memory functions which is associated with increase in brain acetylcholine levels. Present results show that administration of nuts attenuated the buscopan effects. Since there is a lower the risk of degenerative illness via lowering oxidative stress and inhibiting

macromolecules oxidation, naturally occurring antioxidants like phenols are essential and very useful for human wellbeing (Mohammadi et al 2014).

5. CONCLUSION

The outcome of present study is that buscopan (hyoscine butyl bromide) is involved in memory impairment but effects were attenuated by Juglan showing protective feature on health. The same type of results seen in brain acetylcholine levels that strengthen the concept about beneficial features of using nuts in daily life. Moreover, nuts also enhance the antioxidant defense system. Further research needed that walnut consumption over the long run could be particularly advantageous since it improves memory.

6. ACKNOWLEDGMENT

The authors are thankful to the Dean Faculty of Science, Federal Urdu University, for funding this project.

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