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Protective effects of Olive oil on the stress induced behavioral changes in rats

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Abstract: The objective of the current study was to examine protective effects of olive oil having high magnitude of essential components and likely to guard the brain from damaging outcomes of stress. Stress is a key etiological feature in the development of various diseases. Olive oil is a chief constituent of Mediterranean diet, has been used from the past for nutritional and pharmacological purposes. It is a rich source of vital dietary and nutritional constituents with excellent health benefits. Therefore, this study was planned to see the potential role of olive oil on stress-related behavioral shortfalls in rats. Behavioral parameters such as open field test, light and dark activity test, forced swim test, hot plate and water maze test were used for investigations of locomotor activity, level of anxiety, depression, analgesic activity and long term memory in rats respectively. The outcomes of present study displayed increased locomotion, decreased anxiety and depressive behaviors, improvement in long term memory as well as analgesic activity in restraint rats pretreated with olive oil. The results from present study reflected that 2 h restraint stress induced anxiogenic; depressive, nociceptive and memory impairing effects in rats are decreased following repeated administration of olive oil in rats. Our finding highlights the significance of using herbal compounds in the recovery of stress related behavioral impairments as they possess minimal side effects as compared to synthetic drugs.

Key Words: Olive oil, Antidepressant, Anxiolytic, Nociceptive, Anxiogenic, Analgesic
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INTRODUCTION

Stress is not considered as a disease itself but continuous exposure to stressful episodes has been related to the onset and advancement of many physiological and neurological illnesses[1-3]**.** All living organism must keep homeostasis while facing challenging stimuli known as stressors [4] Olive oil taken from a natural herb olive, constitutes different components such as mixed triglyceride, esters of oleic acid, palmitic acid and other fatty acids [5]. It is also contains high amount of antioxidants and vitamins. Phenolic mixtures, polyphenols including esters of tyrosol and hydroxytyrosol (oleocanthal and oleuropein) possess neurodefensive properties [6, 7].Other phenolic constituents include aldehydicsecoiridoids, flavonoids and lignans [8].Olive oil also contains monounsaturated fatty acids and have beneficial effects on health. The long chain omega 3 fatty acid, docosahexaenoic acid (DHA) provides neuroprotection against stress induced neurological impairments, inflammation, and apoptosis[9-11]. High levels of unsaturated fatty acids in serum have protective roles against stress by decreasing the release of pro-inflammatory cytokines [12]. Other component hydroxytyrosol present in olive oil acting as antioxidants have also been reported to defend against markers associated with antherogenic process[13-15]. Stress inducing situations may cause several changes in body and develops alterations in overall behavioral patterns in humans and animals. Extensive studies to investigate behavioral, neurological and biochemical changes following repeated administration of olive oil in various animal models has been described and it was found that olive oil possess analgesic, anxiolytic as well as antidepressant properties [16, 17]. Previous work in our laboratory also elucidated the antidepressant and anxiolytic properties of olive oil and suggested that these effects may be due to changes in neurotransmission levels which were attenuated by the administration of olive oil in rats [18].In view of the previously reported studies on beneficial effects of olive oil, current study was planned to monitor effects of olive oil in animal model of learned helplessness. Therefore, in present study role of olive oil on exploratory activity, anxiety, depression and memory tasks in rats will be monitored.

MATERIALS AND METHODS

Animals

Locally breed male Albino Wistar rats were obtained from the ICCBS, University of Karachi, Pakistan weighing about 150-200 g. Animals were housed separately and allowed access for diet and water one week prior to the start of experiment so that rats get adopted themselves in the environment.

Protocol

The rats were categorized into 2 groups as control and olive oil treated. Each group was given with standard rodent diet. 0.3 ml/day tap water was given orally to control rats and 0.3 ml/day olive oil was administered orally to oil group for 3 weeks, food and water were accessible for 24 hours to each group. Treatment with olive oil continued for 21 days and after this period groups were further

categorized as unrestraint and restraint groups. Restraint group was given stress for 2 h / day for 2 days.

Restraint Procedure

After the completion of olive oil administration protocol, rats of restraint group (control and oil) were subjected to acute restraint stress (2 h /day for 2 days) in ventilated, closed plastic tubes with length of 20 cm and diameter of 6.5 cm that permit only restricted exploration [19]. Unrestraint rats were kept in their home cages throughout the experimentation. Behaviors were monitored after 2 h of stress procedure.

Behavioral Parameters

24h after restraint stress behavioral activities of unrestrained and restrained animals were monitored. All experiments were carried out by the formal approval from the department ethical committee for animal care in agreement with NIH Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Open Field Test (OFT)

OFT was used to monitor locomotion in rats. The method followed in current study was same as described previously [20,21]. The parameter noted to observe exploratory activity were in terms of number of square crossed by rats as described in earlier studies [22].

Forced Swim Test (FST)

FST is the most accepted model for the assessment of depression in animals [23].In the present study the parameter noted was struggling by rats to observe depressive behavior, method was followed same as described and performed earlier in our laboratory [24].

Light / Dark Activity Box

Anxiety level in animal is observed by using light and dark activity box. Time expended in light box was noted, method was same as performed previously in our laboratory [25]. Increased time of stay in light compartment will served as an index of decrease anxiety state in rats.

Hot Plate Test

The method of hot plate test used is same as described earlier [26] The apparatus consist of metal plate surrounded by a transparent cylinder. The metal plate is maintained at a constant temperature which can be varied between 50-55 °C. It seems to produce reliable and fairly stable [25] pain threshold to measure analgesic activity. Animal to be tested is placed in a cylinder on metal plate. Most often the animal initial response is to sit on its hind legs and lick its fore paws. Decrease number of licks served as reduce pain threshold in rats.

Water maze test

Water maze test was performed in a similar manner as reported earlier [27]. Time to reach hidden platform served memory retention in rats.

Statistical Analysis

Data is displayed as mean ± S.D. The software used for data analysis is SPSS Version 20. Two way analysis of variance (ANOVA) was done to understand the results of treatment between groups and Tukey's post hoc analysis was done. The significance level was considered as $p \leq 0.05$.

RESULTS

The beneficial effects of olive oil on behavioral parameters in restraint rats were observed. Locomotor activity was observed by using OFT. In Fig. 1 significant effects of stress ($F= 211.98$; $df=1, 20$; $p<0.01$), olive oil ($F= 168.471$; $df =1, 20$; $p<0.01$), and non-significant relationship among stress and olive oil ($F=2.866$; $df=1, 20$; $p>0.05$) were observed. Tukey's test shows decreases number of square crossed in 2 h restraint stress rats.

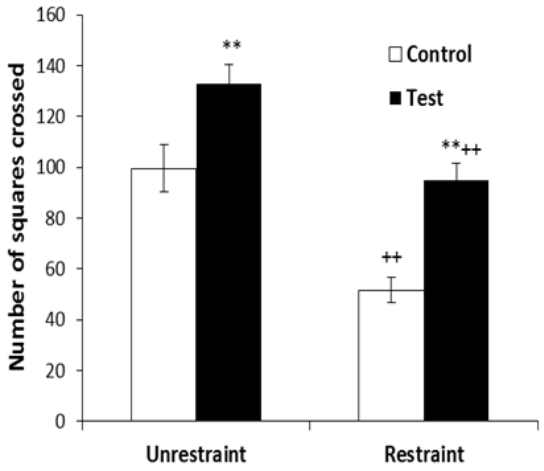

Figure 1: Values are represented with mean + SD (n=6). Tukey's test was performed and significant levels **p <0.01 from their relevant controls (water treated) while, ++p<0.01 from their relevant unrestraint controls.

Olive oil pretreatment significantly ($p < 0.01$) increased number of square crossed in restraint as well as unrestraint rats when compared with control. Anxiolytic effects of olive oil in rats were observed by light/dark transition box. In Fig. 2 significant effects of stress ($F = 78.327$; $df=1, 20$; $p<0.01$), olive oil ($F = 57.232$; $df=1, 20$; $p<0.01$), and non-significant relationship among stress and olive oil ($F=0.722$; $df=1, 20$; $p>0.05$) were observed. Tukey's test shows decrease time spent in light box.

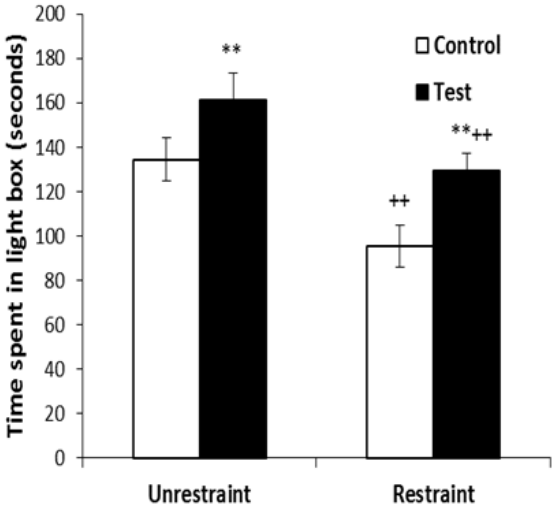

Figure 2: Values are represented with mean + SD (n=6). Tukey's test was performed and significant levels **p <0.01 from their relevant controls (water treated) while, ++p<0.01 from their relevant unrestraint controls.

Olive oil pretreatment significantly ($p<0.01$) increased time spent in light box in restraint and unrestraint rats when compared with respective control. This result shows the anxiolytic effect of olive oil. Forced swim test is suitable to monitor depressive behaviors in rats [28].In Fig. 3 non-significant effect of stress ($F = 3.419$; $df=1, 20$; $p>0.05$), significant effects of olive oil ($F = 313.315$; $df=1, 20$; $p<0.01$), and significant relationship among stress and olive oil ($F=13.435$; $df=1, 20$; $p<0.01$) were observed. Tukey's test shows that 2 h restraint stress significantly ($p<0.01$) decreased struggling time in rats.

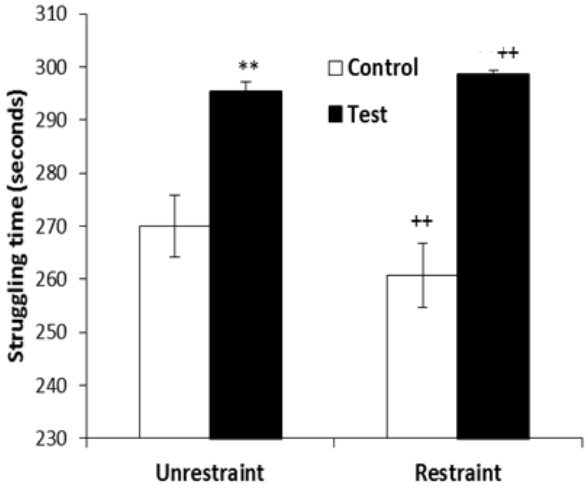

Figure 3: Values are represented with mean + SD (n=6). Tukey's test was performed and significant levels **p <0.01 from their relevant controls (water treated) while, ++p<0.01 from their relevant unrestraint controls.

Olive oil pretreatment significantly increases ($p<0.01$) struggling time in restraint and unrestraint rats when compared with respective control. These results show the antidepressant properties of olive oil. Present study also investigates the analgesic activity of olive oil in rats by using Hot plate test.In Fig. 4 significant effect of stress ($F = 306.816$; $df=1, 20$; $p<0.05$), olive oil ($F = 554.007$; $df=1, 20$; $p<0.01$), and significant relationship among stress and olive oil ($F=145.019.$; $df=1, 20$; $p<0.01$) were observed. Tukey's test shows that 2 h restraint stress significantly ($p<0.01$) increase number of licks in restraint rats.

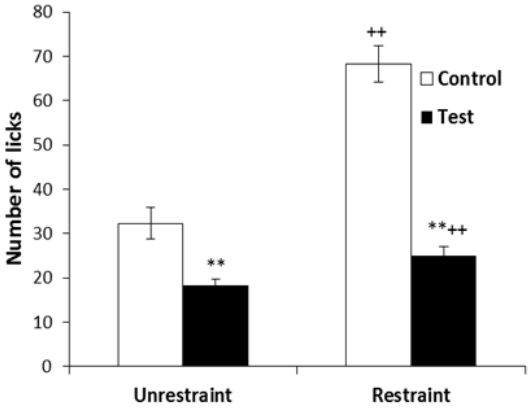

Figure 4: Values are represented with mean + SD (n=6). Tukey's test was performed and significant levels **p <0.01 from their relevant controls (water treated) while, ++p<0.01 from their relevant unrestraint controls.

Olive oil significantly ($p<0.01$) decreased number of licks in restraint rats. The effect of repeated administration of olive oil on memory and learning behaviors was monitored by water maze test. In Fig. 5 non-significant effect of stress ($F= 2.440$; $df=1, 20$; $p>0.05$), significant effect of olive oil ($F= 275.043$; $df=1, 20$; $p<0.01$), and significant relationship among stress and olive oil ($F=13.198$; $df=1, 20$; $p<0.01$) were observed. Tukey's test shows that 2 h restraint stress significantly ($p<0.01$) increases the time to reach the hidden platform control rats. Olive oil pretreatment significantly reduced time to be placed on hidden platform in restraint and unrestraint rats when compared with respective control.

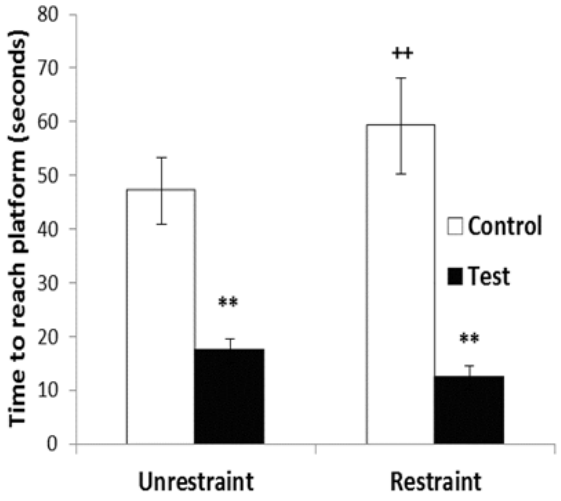

Figure 5: Values are represented with mean + SD (n=6). Tukey's test was performed and significant levels **p <0.01 from their relevant controls (water treated) while, ++p<0.01 from their relevant unrestraint controls.

DISCUSSION

Olive oil is widely used all over the world because of its advantageous health effects. The present study investigated that olive oil pretreatment might be useful in stress induced alterations in behaviors in rats. In present study 2 h restraint stress induced decrease in locomotor activity, increased anxiety and depression with concomitant impairment in memory state as well as increase in nociception were observed. Stress decreases exploratory activity of animals have also been stated earlier [29].However, increased locomotor activity after repetitive administration of olive oil has also been described [28]. The restraint stress induced decrease in locomotor activity as evident by reduced number of squares crossed in OFT and increased anxiogenic behaviors with decrease time spent in light dark activity box were not observed in restraint rats pretreated with olive oil in present study. Changes in neurotransmitter levels contribute in the development of anxiety states. Stress increases serotonin levels in the brain [30], thereby causing fear and anxiety in animals. In present study it is suggested that anxiolytic effects of olive oil may be due to the modifications in 5-HT levels mainly by decreasing 5-HT levels in the brain. Olive oil decreases 5-HT levels were also observed in previous studies [17, 18] . Anxiety and depression are the most prevalent neuropsychiatric illnesses. Antioxidants have ability for removal of stress related generation of reactive oxygen species .Olive oil also serve as antioxidants as they are rich in polyphenol containing compounds oleic acid, tocopherols, oleuropein and unsaturated fatty acids and are suspected to reduce anxiety states in present study. Increased immobility time in FST observed in restraint stress was not observed in olive oil pretreated restraint stress suggesting their antidepressant actions. The ameliorative effects of Mediterranean dietary component containing polyunsaturated fatty acids in depression have been studied previously [31]. In present study the antidepressant potential of olive oil as observed by increased struggling time in restraint rats might be due to the presence of omega 3 fatty acids. Studies in animal models reported that decrease in 5-HT levels contribute to the development of depression. Omega 3 fatty acids increase 5-HT levels and therefore suggested in present study that antidepressant actions of olive oil is due to the omega 3 fatty acids. Similarly linoleic acid, omega 6 poly unsaturated fatty acids present in olive oil also reduced symptoms of depression[18, 32, 33] .Restraint stress induced depression and concomitant decline in cognition were observed in present study as restraint rats took more time to be placed on submerged unseen platform in water maze test. Stress related generation of oxidative species may disrupt neurons leading to memory impairments. It is therefore suggested that polyphenols may play contributing role in the

maintenance of neuronal integrity and improve cognitive and thinking processes. Outcomes of present study consistently support that olive oil improve memory functions and attenuate stress related memory dysfunction in rats [34, 35] . Strong analgesic activity of olive oil has been reported previously [36]. Licking of paws, number of jumps and latency to lick indicates rat's perception to pain. Stress inducing situation releases pain and inflammatory mediators and enhanced nociception [36]. Potential analgesic properties of olive oil has been stated in different animal models [37]. Results from the present study suggested that polyphenols produced analgesia via the inhibition of pain and inflammatory pathways. Analgesic properties of olive oil observed in present study are consistent with several other reported studies[38].

CONCLUSION

Results of present study show that olive oil attenuate stress induced behavioral deficits. Majority of the components present in olive oil are beneficial and provide positive outcomes in altered behaviors due to restraint stress. Results from present study suggested that olive oil exerts peripheral to central actions and it can be used as herbal therapy for various ailments. However, more studies are recommended to explain the particular action by which olive oil produces its effects on behavior.

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REFERENCES

1. Emad S, Qadeer S, Sadaf S, Batool Z, Haider S and Perveen T. Attenuation of stress induced memory deficits by nonsteroidal anti-inflammatory drugs (nsaids) in rats: role of antioxidant enzymes. *Pharmacol Rep*. 2016 (in press).
2. Mc Ewen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res*. 2000; 886: 172-189.
3. Sevgi S, Ozek M, Eroglu L. L-NAME prevents anxiety-like and depression-like behavior in rats exposed to restraint stress. Methods Find *Exp Clin Pharmacol*. 2006; 28: 95-100.
4. Chrousos GP. Stress and disorders of the stress system. *Nat. Rev. Endocrinol*. 2009; 5(7): 374-381.
5. Tuck KL and Hayball PJ. Major phenolic compounds in olive oil: metabolism and health effects. *J. Nutr. Biochem* 2002; 13: 636-644.
6. Abdullah A, Uddin MS, Ferdous Wahid MA and Rahman MM. Neurodefensive effect of *Olea europaea* L. In alloxan-induced cognitive dysfunction and brain tissue oxidative stress in mice: incredible natural nootropic. *J. Neurol. Neur*. 2016. 7:S3
7. Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S and La Guardia M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr Res Rev*.2005; 18: 98-112.
8. Owen R, Giacosa A, Hull W, Haubner R, Spiegelhalder B and Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *Eur. J. Cancer*. 2000; 36: 1235-1247.
9. Gertsik L, Poland RE, Bresee C and Rapaport MH. Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder. *J Clin Psychopharmacol*.2012; 32(1): 61.
10. Bradbury J, Myers SP and Oliver C. An adaptogenic role for omega-3 fatty acids in stress; a randomised placebo controlled double blind intervention study (pilot)[ISRCTN22569553]. *J. Nutr*. 2004; 3: 1.
11. Takeuchi H, Kondo Y, Yanagi M and Yoshikawa M. Accelerative effect of olive oil on adrenal corticosterone secretion in rats loaded with single or repetitive immersion-restraint stress. *J Nutr Sci Vitaminol*.2000; 46: 158-64.
12. Maes M, Christophe A, Bosmans E, Lin A and Neels H. In humans, serum polyunsaturated fatty acid levels predict the response of proinflammatory cytokines to psychologic stress. *Biol. Psychiatry* . 2000; 47: 910-920.
13. Bondia-Pons I, Schröder H and Covas M-Il. Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-Mediterranean participants. *J. Nutr*. 2007; 137: 84-87.
14. Andreadou I, Iliodromitis EK and Mikros E. The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *J. Nutr*. 2006; 136: 2213-2219.
15. Gonzalez-Santiago M, Martin-Bautista E and Carrero J. One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. *Atherosclerosis* 2006; 188: 35-42.
16. Pitozzi V, Jacomelli M and Zaid M. Effects of dietary extra-virgin olive oil on behaviour and brain biochemical parameters in ageing rats. *Br. J. Nutr*.2010; 103: 1674-1683.
17. Gupta A, Vij G and Chopra K. Possible role of oxidative stress and immunological activation in mouse model of chronic fatigue syndrome and its attenuation by olive extract. *J. Neuroimmunol*.2010; 226: 3-7.
18. Perveen T, Hashmi BM, Haider S, Tabassum S, Saleem S and Siddiqui MA. Role of monoaminergic system in the etiology of olive oil induced antidepressant and anxiolytic effects in rats. ISRN *Pharmacol*.2013.
19. Delaney G, Dawe K and Hogan R. Role of nociceptin/orphanin FQ and NOP receptors in the response to acute and repeated restraint stress in rats. *J. Neuroendocrinol*.2012; 24: 1527-1541.
20. Kennett GA, Dickinson SL and Curzon G. Central serotonergic responses and behavioural adaptation to repeated immobilisation: the effect of the corticosterone synthesis inhibitor metyrapone. *Eur. J. Pharmacol*.1985; 119: 143-152.
21. Haleem DJ, Kennett G and Curzon G. Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. *Brain Res*. 1988; 458: 339-347.
22. Pereira P, Tysca D, Oliveira P, da Silva Brum LF, Picada JN and Ardenghi P. Neurobehavioral and genotoxic aspects of rosmarinic acid.*Pharmacol. Res*. 2005; 52: 199-203.
23. Porsolt R, Bertin A and Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther*.1977; 229: 327-336.
24. Khaliq S, Haider S, Naqvi F, Perveen T, Saleem S and Haleem DJ. Altered brain serotonergic neurotransmission following caffeine withdrawal produces behavioral deficits in rats. *Pak J Pharm Sci*. 2012; 25: 21-5.
25. Farooq R, Haleem DJ and Haleem M. Dose related anxiolytic effects of diazepam: relation with serum electrolytes, plasma osmolality and systolic blood pressure (sbp) in rats. *Pak Jour Pharmacol*. 2008; 25: 37-42.
26. Fischer BD, Zimmerman EI, Picker MJ and Dykstra LA. Morphine in combination with metabotropic glutamate receptor antagonists on schedule-controlled responding and thermal nociception. *J. Pharm.Exp. Ther*. 2008; 324: 732-739.
27. Khaliq S, Irfan B, Haider S and Haleem DJ. M-CPP induced hypolocomotion does not interfere in the assessment of memory functions in rats. *Pak J Pharm Sci*. 2008; 21: 139Á43.
28. Khaliq S, Haider S and Tabassum S. Behavioral effects following administration of olive oil in rats. *Asian j. Pharm. Biol Res*.2012; 2.

29. Haleem DJ and Parveen T. Brain regional serotonin synthesis following adaptation to repeated restraint. *Neuroreport* 1994; 5: 1785-1788.
30. Logan AC. Neurobehavioral aspects of omega-3 fatty acids: possible mechanisms and therapeutic value in major depression. *Altern Med Rev*.2003; 8: 410-425.
31. Grosso G, Galvano F and Marventano S. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxid Med Cell Longev*. 2014.
32. Sirtori CR, Anderson JW and Arnoldi A. Nutritional and nutraceutical considerations for dyslipidemia. *Future Lipidology*. 2007: 2; 313-339.
33. Smith MA, Beilin LJ, Mori TA and Oddy WH. Essential fatty acids and mood: a systematic review of observational studies. *Am J Food Nutr* 2011; 1: 14-27.
34. Haider S, Khaliq S, Ahmed S and Haleem D. Long-term tryptophan administration enhances cognitive performance and increases 5HT metabolism in the hippocampus of female rats. *Amino Acids* 2006; 31: 421-425.
35. Haider S, Shameem S, Ahmed SP, Perveen T and Haleem DJ. Repeated administration of lead decreases brain 5-HT metabolism and produces memory deficits in rats. *Cell Mol Biol Lett.*, 2005; 10: 669.
36. Fezai M, Senovilla L, Jemaà M, Ben-Attia M. Analgesic, anti-inflammatory and anticancer activities of extra virgin olive oil. *J Lipids*. 2013; doi.org/10.1155/2013/129736.
37. Eidi A, Moghadam-kia S, Moghadam JZ, Eidi M and Rezazadeh S. Antinociceptive and anti-inflammatory effects of olive oil (*Olea europeae* L.) In mice. *Pharm Biol*.2012; 50: 332-337.
38. Laaboudi W, Ghanam J, Aissam H, Merzouki M and Benlemlih M. Anti-inflammatory and analgesic activities of olive tree extract. *Int. J. Pharm. Pharm. Sci*. 2016; 8:7.