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Anxiolytic and memory enhancing potential of aloe vera and flax seed oil in rats: A comparative study with valproic acid

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Abstract: For centuries, herbs and herbal oils are used for pharmacological purpose. Aloe vera is well-known as silent healer and flax seed oil is known to contain rich amount of omega-3 fatty acids, both are having effects on central nervous system. Valproic acid is anticonvulsant drug with some side effects and has shown effects on behaviors. This study was designed to monitor the effects of valproic acid, aloe vera and flax seed oil on cognitive and anxiolytic behaviors in rats. Animals were categorized into four groups: Control, valproic acid, aloe vera and flax seed oil which were respectively treated with water, valproic acid (300mg/kg), aloe vera (0.4ml/kg) and flax seed oil (1.8ml/kg). The treatment was continued 2 weeks for drug and 3 weeks for aloe vera and flax seed oil. Anxiolytic effect as well as increased GABA levels were observed following drug and oil treatments. Improvement in cognitive function with decrease in acetylcholine esterase activity in aloe vera and flax seed oil while impairment in learning memory with increase acetylcholine esterase activity was observed in rats treated with valproic acid. Results showed substantial decrease in acetylcholinesterase level in aloe vera and flax seed oil supporting the cognitive impact of oils in contrary to drug.

Keywords: Valproic acid, aloe vera, flax seed oil, cognition, anxiety, GABA.

INTRODUCTION

Valproic acid (2-propylpentanoic acid) is eight-carbon branched chain fatty acid with unique anticonvulsant properties, it is derived from valeric acid which is naturally produced by valerian, (*Valeriana officinalis*) and usually used as its sodium salt (Chiu *et al*., 2013). It is an antiepileptic drug which has been utilized for more than 3 decades for treating partial and generalized seizures (Lheureux *et al*., 2005). It is also used for treating bipolar disorder (Chiu *et al*., 2013). Valproic acid (VPA) inhibits the gamma-amino butyric acid (GABA) transaminase and thereby alters level of GABA in brain (Rogawski and Loscher, 2004). Along with the antiepileptic properties, VPA is known to have effects on learning and memory. Several reports have been documented on moderate to mild range of cognitive impairments and memory deficits in patients taking VPA (Umka *et al*., 2010, Senturk *et al*., 2007). Aloe-vera (*Aloe barbadensis*) is also called the healing-plant or silent healer (Choi and Chung, 2003). It contains multiple active constituents such as anthraquinones, amino acids, vitamins, lignin, sugar, salicylic acid, saponins and minerals (Park and Jo, 2006) having antibacterial, anti-inflammatory, antidiabetic and antilipidemic activity. Aloe vera has shown therapeutic effects against epilepsy, Alzheimer's disease, depression and anxiety in previous studies (Baruah *et al*., 2016; Foster, 1999). Aloe vera oil is acquired from its gel and has all its vitamins, mineral salts and carbohydrates (Badke *et al*., 2019; Maan *et al*., 2018). Flax (*Linum usitatissimum*) is the member of Linaceae family and has extensive history of been conventionally used as a fiber and source of oil. Flax seed oil (FSO) is used worldwide for its potential health benefits. It is known to have bactericidal, anticancer, anti-atherogenic, anti-inflammatory laxative effects (Varghese *et al*., 2017; Zuk *et al*., 2011). FSO is richest plant source having omega-3 fatty acid mostly alpha-linolenic acid (ALA), protein, dietary fibers and lignans (Wang *et al.,* 2017; Kajla *et al*., 2015). Studies have reported that omega-3 fatty acid also have protective cognitive effects (Fernandes *et al*., 2011; Singh, 2005) and larger amount of docosahexaenoic acid (DHA) in brain help to increase learning ability (Hashimoto *et al*., 2005).

In view of above findings, present study was designed to investigate the behavioral changes that appear following long term administration of aloe vera and flax seed oil and compare these changes with effects produced by anticonvulsant drug VPA in rats.

MATERIALS AND METHODS

Experimental Protocol

Animals

Locally breed Albino Wistar rats were procured HEJ Research Institute of Chemistry, Karachi, Pakistan, with average weight of 150-200g. To circumvent the social interaction effects animals were caged individually in a quiet room of controlled temperature (22±2ºC) with free

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access of water and rodent food so they can accustom themselves.

Plant materials and drug

Aloe vera oil and Flax seed oil were purchased from a herbal shop. Valproic acid used in this study was purchased from Sigma Aldrich Company USA.

Grouping and dosing protocol

For this study, twenty-four rats were categorized randomly into Control, Valproic acid, Aloe vera and Flax seed oil (n=6) groups. During the study, control group were given water. Rats of aloe vera oil group received oral dose of 0.4ml/kg aloe vera oil (Rathore *et al*., 2014). The flax seed oil group received oil orally at dose of 1.8ml/kg (Tanna *et al*., 2012). The administration of drugs was continued to respective group for 3 weeks. While the valproic acid treated group received 300mg/kg of intraperitoneal dose for 2 weeks (De *et al*., 2017). The animals were then subjected to evaluate behavioral changes and were decapitated afterwards. The samples of plasma and brain were collected and stored at -70°C for biochemical estimations. Institutional Review Board (IRB) formal approval ref no: 03364/SC-2015 was granted before performing the experiment. The guidelines issued by National Institute of Health (NIH) Care and Use of laboratory animals were followed strictly (Publication No. 85-23, revised 1985).

Behavioral analysis

Open field test (OFT)

The ambulatory activity is monitored in OFT. The method was similar as performed before in our laboratory (Naqvi *et al*., 2012).

Light/ dark transition test (LDT)

In LDT test we observed the time animal spent in light area. This test was performed to monitor the anxiogenic / anxiolytic behavior of the rats. The method and apparatus was similar as described earlier (Khaliq *et al*., 2012).

Passive avoidance test (PAT)

PAT is a fear conditioning test, the rats learning memory was observed in order to avoid the aversive stimulus (Tabassum *et al*., 2017). The apparatus and method was same as described in earlier studies (Haider *et al*., 2016).

Novel object recognition test (NORT)

This test is performed to evaluate the memory and recognition response of rats (Ennaceur and Delacour, 1988). The paradigm and method was same as described previously (Haider *et al*., 2016).

Elevated plus maze (EPM)

Short and Long term

EPM was performed to evaluate the cognitive behavior of rodents by observing the time taken to enter the closed arms. The method and apparatus was same as described before (Anis *et al*., 2016).

Acetylcholinesterase estimation

Acetylcholinesterase of whole brain was estimated as described previously (Batool *et al*., 2016).

GABA estimation

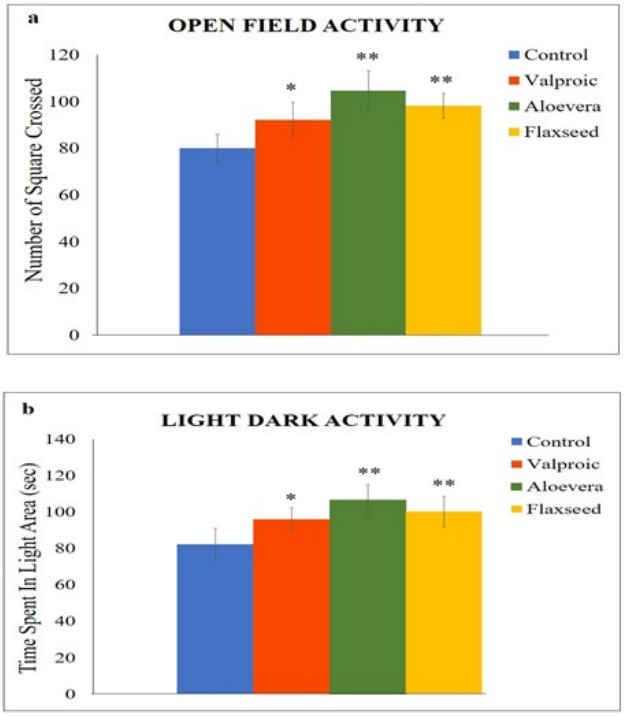
The estimation of GABA was done by ELISA method. Commercially available GABA ELISA kit was purchased from Bioassay Technology Laboratory, China. The optical density was read at 450nm. Method and sample preparation was same as described earlier (Tabassum *et al*., 2017).

STATISTICAL ANALYSIS

Data is represented as mean ± SD. For data analysis one-way ANOVA and Tukey's test was performed in SPSS software (version 20.0), p> 0.05 was consider as non-significant result.

RESULTS

Effects of Valproic acid, Aloe vera oil and Flax seed oil administration on the behavioral activity of rats.

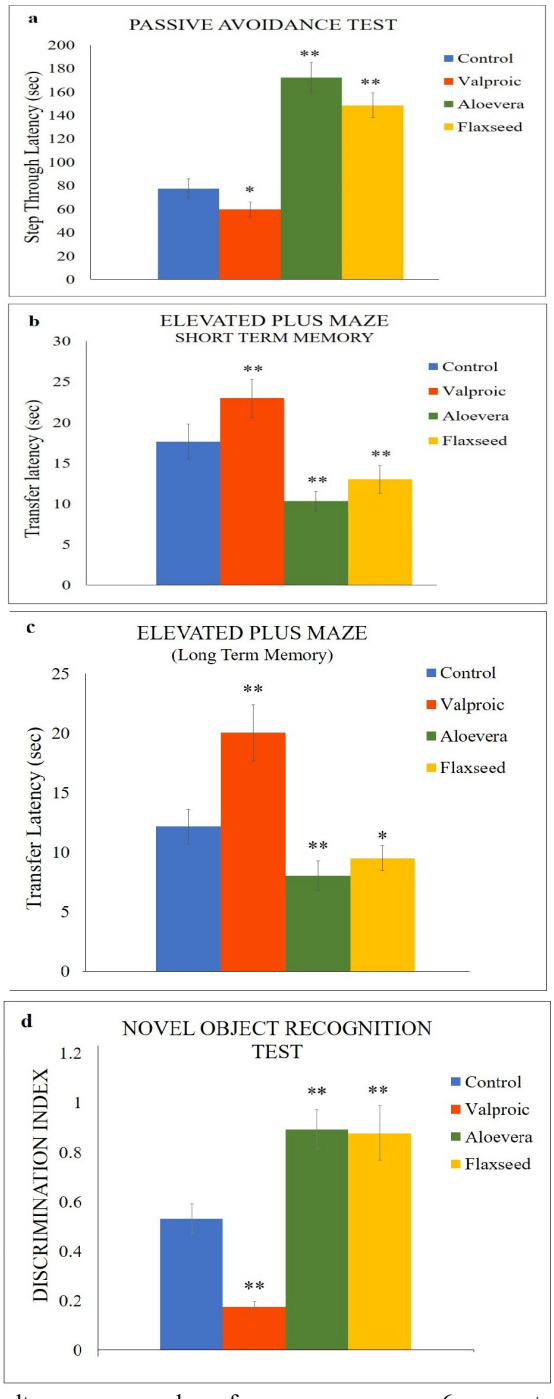


Results are expressed as, for every group n = 6; mean ± SD. Significant difference was calculated by Tukey's test *($p<0.05$) and **($p<0.01$) from control.

Fig. 1: Effect of VPA, Aloe vera and FSO on OFT and LDT in rats.

Fig. 1a shows the effect of valproic acid, aloe vera oil and flax seed oil on the locomotor activity of rats in OFT.

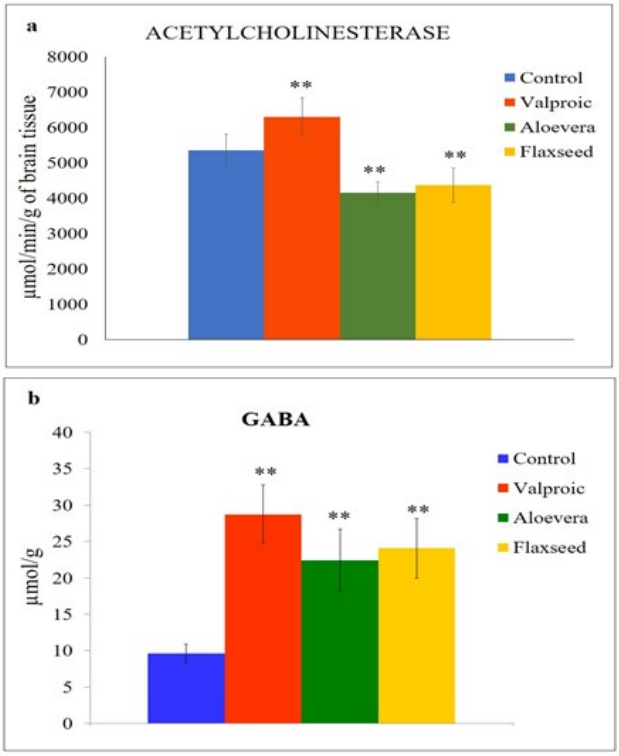
Data analyzed by one-way anova represented significant effects of valproic acid, aloe vera and flax seed oil (df = 3, 20; F=13.532; p<0.01). Post hoc evaluations by Tukey's test showed that the number of square crossed is significantly increase (p<0.01) in the aloe vera oil and flax seed oil treated rats and (p<0.05) in valproic acid treated rats.



Results are expressed as, for every group n = 6; mean ± SD. Significant difference was calculated by Tukey's test *(p<0.05) and **(p<0.01) from control.

Fig. 2: Effect of VPA, Aloe vera and FSO on cognitive function in rats.

Fig. 1b shows the effect of valproic acid, aloe vera oil and flax seed oil on the anxiety in light/dark transition test. Data analyzed by one-way anova showed significant effects of valproic acid, aloe vera and flax seed oil (df = 3,20; F = 9.815; p<0.01). Post hoc evaluations by Tukey's test showed that the time spent in light area is significantly increase (p<0.01) in the aloe vera oil and flax seed oil treated rats and (p<0.05) in valproic treated rats.



Results are expressed as, for every group n = 6; mean ± SD. Significant difference was calculated by Tukey's test *(p<0.05) and **(p<0.01) from control.

Fig 3: Effect of VPA, Aloe vera and FSO on AChE and GABA levels in rats.

Fig. 2a shows the effect of valproic acid, aloe vera oil and flax seed oil on the memory in PAT. Data analyzed by one-way anova represent a significant effect of valproic acid, aloe vera oil and flax seed oil (df =3,20; F=182.650; p<0.01). Post hoc analysis by Tukey's test evaluated that the step through latency is significantly increase (p<0.01) in the aloe vera and flax seed oil treated rat and significant decrease (p<0.05) in the step through latency of valproic treated rats.

Fig. 2b shows the effect of valproic acid, aloe vera oil and flax seed oil on the short-term memory in EPM test. Data analyzed by one-way anova represent a significant effect of valproic acid, aloe vera oil and flax seed oil (df=3,20; F =51.131; p<0.01). Post hoc evaluation by Tukey's test represent that the transfer latency is significantly decrease (p<0.01) in the aloe vera oil and flax seed oil treated rats

and significant increase ($p<0.01$) in transfer latency was reported in valproic acid treated rats.

Fig. 2c shows the effect of valproic acid, aloe vera oil and flax seed oil on the long-term memory in EPM test. Data analyzed by one-way anova showed a significant effect of valproic acid, aloe vera oil and flax seed oil ($df = 3, 20$; $F = 65.414$; $p<0.01$). Post hoc evaluation by Tukey's test represent that the transfer latency is significantly decrease ($p<0.01$), ($p<0.05$) in the aloe vera oil and flax seed oil treated rats respectively. Significant increase was observed ($p<0.01$) in transfer latency in valproic acid treated rats.

Fig. 2d shows the effect of valproic acid, aloe vera oil and flax seed oil on discrimination index in novel object recognition test. Data analyzed by one-way anova showed a significant effect of valproic acid, aloe vera and flax seed oil ($df=3,20$; $F=121.642$, $p<0.01$). Post hoc evaluations by Tukey's test showed that discrimination index is significantly increased ($p<0.01$) in aloe vera oil and flax seed oil treated rats and significant decrease ($p<0.01$) in discrimination index was seen in valproic acid treated rats.

Fig. 3a shows the effect of valproic acid, aloe vera oil and flax seed oil on acetylcholinesterase. Data analyzed by one-way anova shows significant effects of valproic acid, aloe vera and flax seed oil ($df=3,20$; $F=43.294$; $p<0.01$). Post hoc evaluations by Tukey's test shows significant increase in acetylcholinesterase level ($p<0.01$) in rat brains treated with valproic acid and significant decrease in acetylcholinesterase level ($p<0.01$) in rats treated with aloe vera oil and FSO.

Fig. 3b shows the effect of valproic acid, aloe vera and flax seed oil on brain GABA levels. Data analyzed by one-way anova shows significant effects of valproic acid, aloe vera and FSO ($df=3,20$; $F=29.770$; $p<0.01$). Post hoc evaluations by Tukey's test shows significant increase ($p<0.01$) in rat brains treated with valproic acid and similar significant increase of GABA level ($p<0.01$) in aloe vera and FSO treated rats.

DISCUSSION

Valproic acid is a well-known anticonvulsant and also has mood stabilizing ability. The effect of its chronic high dose administration on learning and memory is quite evident further, it has alternating impact on anxiety. In the current study, it is observed that chronic treatment of aloe vera and flax seed oil have ameliorated effects on memory and anxiety, whilst valproic acid administration results in significant cognitive impairment along with its anxiolytic effects. In the current study, aloe vera and flax seed oil treated rats showed increased step through latency as compared to valproate treated rats in passive avoidance test. EPM was used for the evaluation of short and long term memory and in novel object recognition test, valproate treated rats took increased time to enter the close arms and failed to discriminate the objects, respectively as compared to FSO and Aloe vera treated rats. These cognitive changes may be due to the generalized neuro-suppressant effects of valproate (Umka *et al*., 2010). The generation of new neuron in hippocampus continues during the entire life (Ehninger and Kempermann, 2008; Abrous *et al*., 2005). Acetylcholinesterase level has been monitored in this study to assess the cognitive function. Significant increase in the brain acetylcholinesterase levels has been observed in VPA treated rats. However, AChE levels were decreased in rats treated with FSO and aloe vera oil. It has been observed previously that VPA increase the acetylcholinesterase level and in vitro model it is known to increase acetylcholinesterase activity in rats' brain cell aggregate cultures (Varela *et al*., 2013; Schilter *et al*., 1995). It is therefore suggested that impairment and improvement in cognitive functions following long term administration of VPA and oils respectively is associated with AChE levels. Aloe vera has shown to improve the learning and memory as it reduces oxidative damages mediated by the free radical in cerebral cortex and hippocampus (Halder *et al*., 2013; Parihar *et al*., 2004). Arachidonic acid and docosahexaenoic acid content have shown to associate with better spatial memory performance thus evidencing the memory enhancing property of FSO as it contain high concentration of these poly unsaturated fatty acid.

The light dark test (LDT) was performed to assess the anxiolytic effects of drug and the herbal oils. The aloe vera, FSO and valproic acid treated rats spent more time in the light area as compared to control rats showing the better performance ability as compared to the control rats. These results indicate that aloe vera and FSO have more precise ability to deal with the anxiety as compared to valproic acid treated rats. Moreover, open field test (OFT) was also performed to strengthen the validity of anxiolytic effects of drug and herbal oils. Aloe vera, flax seed oil and VPA have anxiolytic effects as observed by increased number of square crossed in open field test, however rats treated with aloe vera and flax seed oil show more anxiolytic activity than VPA. The significant increase in the GABA levels when treated with aloe vera, FSO and valproic acid is observed. It is reported that GABA is considered as a promising candidate in modulating the responses related anxiety (Nuss, 2005). Studies in vivo have shown that infusion of GABA or GABA receptor agonist and antagonist have altered the anxiety responses like anxiolytic and anxiogenic effects respectively (Nuss, 2005; Barbalho *et al*., 2009). Increase in GABA levels and anxiolytic effect in rats treated with VPA, Aloe vera and FSO is observed in this study. It is therefore suggested that anxiolytic effect following the repetitive

administration of VPA and oils observed in the present study is associated with increased level of GABA.

CONCLUSION

It is concluded that prolong use of anticonvulsant VPA impaired cognitive functions however improvement in memory was observed following FSO and aloe vera oil treatment. These memory impairment and improvement is associated with alteration in acetylcholinesterase activity. Moreover, the anxiolytic effect following VPA, aloe vera and FSO is associated with increased GABA levels. In view of all these findings it is therefore suggested that deleterious effect of VPA produce during the treatment of epilepsy can be reduced by the use of Aloe vera oil / FSO in daily life.

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