ISSN No:-2456-2165

Anxiolytic Effect of 6-Shogaol Protect against Ethanol-Withdrawal Induced Anxiety Like Behavior in Albino Mice

Dhyanendra Singh Baghel*, Dr. Vishal Gupta
Faculty of Pharmacy- Mansarovar Global University,
Gadiya, Sehore, Madhya Pradesh, India

Abstract:- Alcoholism is a general behavioral disorder with excessive intake of alcohol; it results the dependency of alcohol with aversive symptoms upon alcohol withdrawal. Withdrawal from chronic ethanol exposure causes anxiety like symptoms, like: vomiting, nausea, mental confusion, tremors, excessive sweating, ataxia, increased heart rate and convulsion like symptoms. Ethanol withdrawal has been postulated to be associated with specific molecular mechanisms and neuroadaptive changes that may lead to an increased and persistent anxiety state. In this study, investigate the effect of 6-Shogaol in ethanol-dependent mice using Fluoxetine as a control. Measures made in this model were consistent with literature data it suggested that the ethanol dosing daily basis ranging from up to 24 to 30 g./kg yielding ethanol blood level close to 2 g/l (43 mM) produced the emergence of symptoms such as hyperexcitability and heightened anxiety due to ethanol treatment cessation in mice. This report shows that ethanol-withdrawal on chronic administration decreases the no. of entries of mice in the light area, and acute as well as chronic treatment with 6-Shogaol dose dependently reverses their response.

Keywords:- *Alcoholism, alcohol withdrawal syndrome, anxiety, Depression, Social interaction test, elevated plus maze test, light and dark paradigm, mice.*

I. INTRODUCTION

Alcoholism is a general behavioral disorder with excessive intake of alcohol; it results the dependency of alcohol with aversive symptoms upon alcohol withdrawal[1]. Depending on various modulating factors like: environmental experience, genetic predisposition, social context, pharmacological history and other ethanolic consumption can become compulsive and an addictive behavior may evolve [2]. The alcohol withdrawal syndrome shows when an individual reduced or stop the uses of alcohol after prolonged use of alcohol. The withdrawal syndrome is miserly interrupted the central nervous system being in hyper excitability state. The ethanol withdrawal syndrome may include seizures and delirium tremens and also leads to extra-neurotoxicity [3].

Withdrawal from chronic ethanol exposure causes anxiety like symptoms, like: nausea, vomiting, tremors, convulsion, sweating and increased heart rate [4]. The physical symptoms of ethanol-withdrawal shown in rats, which are similar to those seen in humans [5, 6], alcohol withdrawn rats and mice also display increased anxiety-like behavior in the elevated plus maze test, acoustic startle test, and social interaction test [7]. Ethanol withdrawal has been postulated to be associated with specific molecular mechanisms and neurological changes that may lead to increased anxiety state [8]. The central nervous system is markedly affected by acute alcohol consumption. Acute alcohol cause sedation and relief of anxiety, And at higher concentration ataxia, impaired judgement, slurred speech, ataxia and disinhibited behavior, a condition usually called intoxication or drunkenness. (Table-1)

Blood Alcohol Concentration (mg/DL)	Effect of alcohol
50 to 100	Sedation, increased functioning times
100 to 200	Impaired motor function and speech, ataxia
200 to 300	Emesis, stupor
300 to 400	Coma
More than 500	Respiratory depression, death

Table 1: -Blood Alcohol Concentration (BAC) and Clinical Effects in Non tolerant Individuals.

II. MATERIAL AND METHOD

A. ANIMALS

Adult male albino mice (22-25 g.) were housed in the grouped (n=06) and maintain under a standard 12 hr. Light/dark cycle and controlled conditions of temperature and humidity. Mice were purchased from N.I.N., Hyderabad, India. The animal study was performed as per IAEC guideline (Reg. No. 831/BC/04/CPCSEA), constituted for the purpose of control and supervision of experimental animals by the ministry of environment and forest, government of India, New Delhi, India. All experiments was carried out in a systematic order with respect to the treatment condition in the noise free room.

B. DRUG AND CHEMICALS

The 6-Shogaol (>95% purity by HPLC, Molecular weight 470.61) was purchased from natural remedies ltd., Bangalore, India. And was stored at 20°C. Fluoxetine was purchased from Cadila pharmaceutical and ethanol was purchased from Mark, India. Fresh solution was prepared before the start of the experiments.

ISSN No:-2456-2165

C. DOSAGE

According to our first round studies with different dosages (10 mg/kg, 20 mg/kg and 30 mg/kg) of 6-shogaol, it was found that the higher dose of 6-shogaol (30 mg/kg) produced a significant effect on the ethanol withdrawal syndrome. Hence 30 mg/kg body wt dosage was considered for this study.

D. ETHANOL-WITHDRAWAL STATE:

During the first time period, all singly housed animals received a liquid diet (40 ml/day at 08:00 a.m.) for 7-10 days ad libitum to habituate them to these sole food and fluid sources. The liquid diet consisted of chocolate milk supplemented with 5 g/L of minerals and vitamin mixture (Profeed; Syncom health care Ltd., Mumbai). Mice consumed 900-1100 g/kg/day over this period. There were no differences observed in the weights of animals at the end of this experimental period. During the second time period, the ethanol administration procedure described by verley et al.,[49] with slight modifications was used. The ethanol treated mice received a liquid diet containing 3% (volume/volume) of ethanol for 8 days, then after this replaced the diet with 4% (volume/volume) ethanol diet for 7 days. The control mice received chocolate diet for all 15 days. No other extra feed or water was supplied over this period and all animals had unlimited access to the diet. At the day 15 at 08:00 am., Alcohol chocolate diet was replaced by nonalcoholic diet by the use of animals in the different experiments. Separate group of animals was used for each set of experiments.

III. BEHAVIORAL ACTIVITY

A. MEASUREMENT OF THE WEIGHT OF MICE BEFORE AND AFTER ETHANOL-WITHDRAWAL:

Before the starting to experiment first of measure, weight of all animals then after the administration of ethanol on experimental groups observe continuously the weight of all the animals, and note down the weight of each animal continuously as per record.

B. INFLUENCE OF ETHANOL-WITHDRAWAL ON BEHAVIORAL ACTIVITY IN MICE:

Light and dark test, and Elevated Plus Maze (EPM) was assessed at 0, 6, 24, 48, and 96 h time interval after ethanol-withdrawal. The time interval at which mice exhibited light area was recorded in experimental (ethanol diet) group. The locomotor activity was recorded simultaneously.

C. LIGHT AND DARK TEST:

The light and dark test were, as per the design by Verley et al. (2009)[49] with slight modifications of paradigm. This test makes use of the rodent's natural disinclination to bright areas as compared to a darker area. In the two-compartment light and dark box appratus, The mice prefer Dark area and should hesitate to enter into light area. The apparatus is a rectangular box and their size is 46x 27x 30 cm, divided into a small area (18x27cm) and a broad area (27x 27cm) with an opening door (7.5x7.5 cm) located in the center of the partition at basement side. The close-topped small compartment applied black paint and fix a dim red light 6W, whereas the open-topped big compartment is painted white and brightly illuminated by a 60W light source. The compartments are equipped with infrared beam sensors enabling the detection of locomotion in each zone, latency of the first crossing from one compartment to the other and shuttle crossings between both compartments. The test was conducted in a sound-attenuated room, under a light intensity of 400-500 Lux. Mice were placed individually in the middle of the light area facing the opening. A 5-min test was given during which the latency to enter the brightly lit area with all four paws, the number of crossings in the white compartment, and the number of transitions between the two compartments was recorded. The floor of each box was cleaned with 10% ethanol between sessions. 6-Shogaol (10 and 30 mg/kg) and Fluoxetine (10 and 30 mg/kg) were administered p.o. 30 min, respectively, before the test for Acute study and twice daily for chronic study. Control animals received an equivalent volume of corresponding vehicle.

D. ELEVATED PLUS-MAZE TEST (EPM):

The EPM test was performed as previously deviously discussed parameter [50,51]. The elevated plus maze apparatus resembled like a plus symbol (+) like shape, as per their shape that name was derived. The elevated plus maze apparatus contains two open arms and two closed arms, that extended from a common central platform. A small raised wall around the edges of the open arms helped to prevent mice from slipping off from that side. The apparatus was constructed from polypropylene and Plexiglas, with a white floor and clear walls, and elevated to a height of 38 cm above floor level. After dosing of the drug, the mouse was placed on the center of the apparatus. And allowed to freely movement of animals under a light intensity of 200 LUX for 05 min. The apparatus was cleaned with 70% ethanol solution after the completion of one phase of the experiment performed. Observe the behavioral activity of mice and scored entries of close (an arm entry was defined as all four paws into an arm) and the time spent in the open arm.

IV. RESULT

- **Effect of 6-Shogaol on mice behavior after the withdrawal of acute and chronic ethanol.**

A. MEASURMENT OF THE WEIGHT OF MICE BEFORE AND AFTER ETHANOL-WITHDRAWAL:-

The records of the weight measurement are shown that, weight of animals are raised very fast after the ethanol withdrawal as compared to the normal weight, but 6-Shogaol (30 mg/kg.) and Fluxetine(30mg/kg.) prevent the excessive weight increment of mice. Two-way ANOVA followed by bonferroni test was performed that in the ethanol-withdrawal state, the weight variation was significantly higher at 1, 5, 10 and 15th day interval compared to control (sucrose diet) group but test drug controlled it. [F (3, 80) = 30.66, p< 0.0001].

ISSN No:-2456-2165

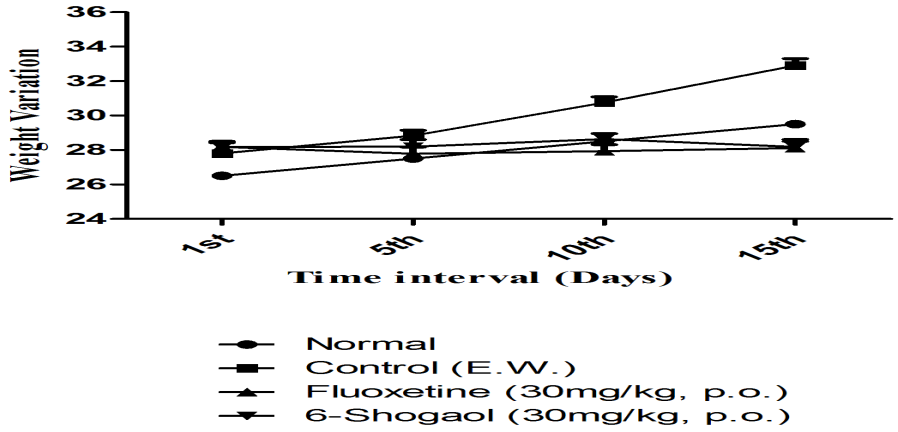

Fig.1: Influence of ethanol-withdrawal on Weight variation in mice: ethanol-treated mice received a diet containing 3% (vol/vol) ethanol for 8 days then a diet containing 4% (vol/vol) ethanol for 7 days. Control mice received the same chocolate diet. On day 1st, 5th, 10th and 15th of experiment measure the weight of all mice individually. Values are expressed as mean±S.E.M (n = 6). Values are statistically significant at *p < 0.001 vs. respective control group (Two-way ANOVA followed by Bonferroni test).

B. INFLUENCE OF ETHANOL-WITHDRAWAL ON BEHAVIORAL ACTIVITY IN MICE:

Two-way ANOVA followed by bonferroni test was performed that in the ethanol-withdrawal state, the light and dark test was significantly higher at 6, 24, 48 and 96 h time interval compared to control (sucrose diet) group with its peak at 24 h time interval [F (4, 10) = 96.89, p< 0.0001] (Fig.-2). And the EPM test was also significantly higher at 6, 24, 48 and 96 h time interval compared to control group with its peak at 24 h time interval [F (4, 10) = 44.83, p< 0.0001].

However, locomotor activity in the ethanol - withdrawal state was unaffected. Two-way ANOVA revealed is an insignificant ethanol-withdrawal effect [F (4, 10) = 0.38, p=0.5500].

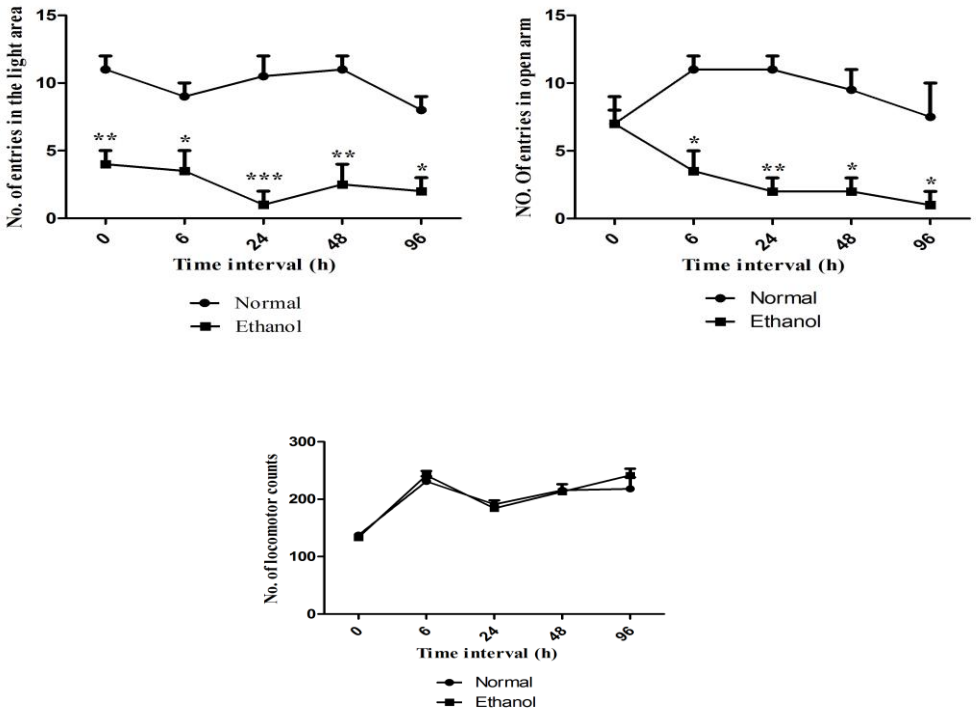

Fig. 2: Influence of ethanol-withdrawal on light & dark test and EPM in mice: ethanol treated mice received a diet containing 3% (vol/vol) ethanol for 8 days than a diet containing 4% (vol/vol) ethanol for 7 days. Control mice received the same chocolate diet. On day 15th, ethanol was withdrawn and the Light & dark test and EPM along with locomotor activity was assessed at 0, 6, 24, 48, and 96 h intervals. The values are expressed as mean±SEM (n=6). Values are statistically significant at *p <0.001 vs. respective control group (Two-way ANOVA followed by Bonferroni test).

.

ISSN No:-2456-2165

C. Light and Dark test:

One-way ANOVA followed by Bonferroni test revealed that acute treatment with 6-Shogaol (10 & 30 mg/kg, p.o.), dose dependently peak increase in the light and dark model in ethanol-withdrawal state [F (2, 15) = 23.07, p< 0.0001] as shown in Fig.-3. Fluoxetine (10 & 30 mg/kg, p.o.) had a similar effect [F (2, 15) = 153.5, p< 0.0001].

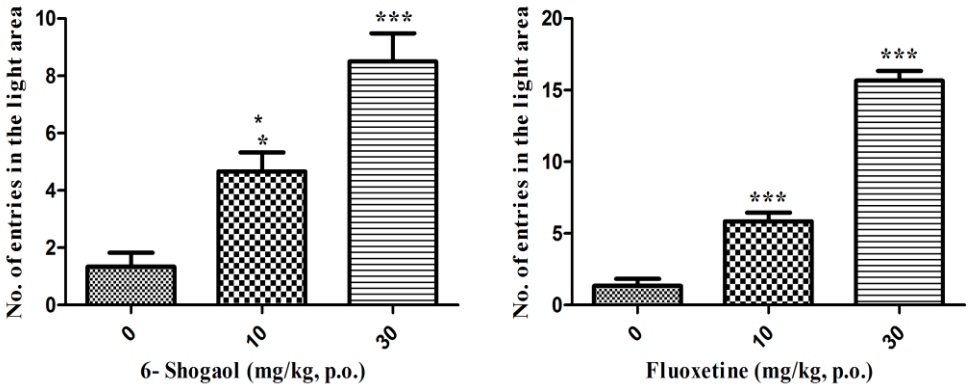

Fig. 3: Effect of acute treatment with 6-Shogaol or Fluoxetine on Light and dark test after ethanol withdrawal: On day 15, 24h after ethanol-withdrawal, experimental (ethanol diet) groups were treated with 6-Shogaol (10 & 30 mg/kg, p.o.) or fluoxetine (10 & 30 mg/kg, p.o.) or vehicle, and after 30 min, Light and dark activity of individual mouse was assessed. Values are expressed as mean±S.E.M (n = 6). Values are statistically significant at *p <0.001 vs. respective control group (One-way ANOVA followed by Bonferroni test)

Two-way ANOVA followed by Bonferroni test revealed that cronic treatment with 6-Shogaol (10 and 30 mg/kg, p.o.) to experimental (ethanol diet) group, significantly (p<0.05) increased the no. of entries in light area evident at 6, 24, and 48h time interval after ethanol-withdrawal. Two-way ANOVA revealed a significant effect of 6-Shogaol treatment [F (3, 20) = 106.89, p< 0.0001] (Fig.-4). Fluoxetine (10 and 30 mg/kg, p.o.) had also a significant effect of light and dark test [F (3, 20) = 116.64, p< 0.0001].

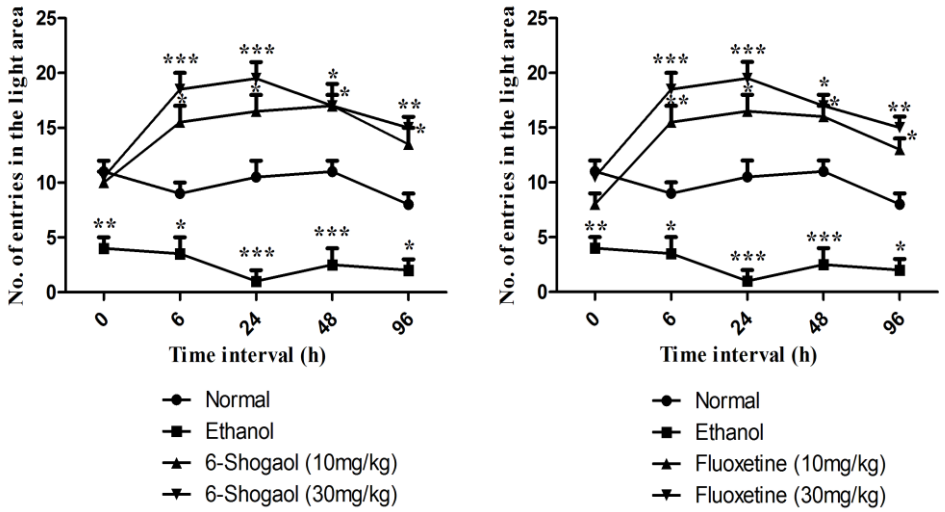

Fig. 4: Effect of chronic treatment with 6-Shogaol or fluoxetine on light and dark test after ethanol-withdrawal: Experimental (ethanol diet) groups were treated with 6-Shogaol (10 and 30 mg/kg, p.o.) or fluoxetine (10 and 30 mg/kg, p.o.) or vehicle twice daily. Control group was daily treated with liquid diet (40 ml/day at 08:00 a.m.). On the 15th day, ethanol was withdrawn; light and dark test of individual group of mouse was examined at 0, 6, 24, 48, and 96 h time intervals. Values are expressed as mean±S.E.M (n = 6). Values are statistically significant at *p <0.05 vs. respective control group, p<0.05 vs. respective vehicle treated experimental group (Two-way ANOVA followed by Bonferroni test).

D. Elevated Plus-Maze (EPM):

One-way ANOVA followed by Bonferroni test revealed that acute treatment with 6-Shogaol (10 and 30 mg/kg, p.o.), dose dependently peak increase in the EPM model in ethanol-withdrawal state shows significant effect [F (2, 15) = 52.77, p< 0.0001]. Fluoxetine (10 and 30 mg/kg, p.o.) had a similar effect [F (2, 15) = 36.08, p< 0.0001].(Fig.-5)

.

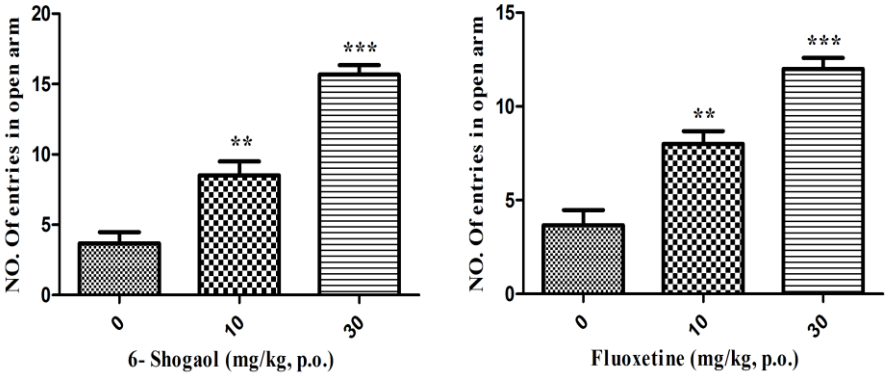

Fig. 5: Effect of acute treatment with 6-Shogaol or Fluoxetine on EPM test after ethanol withdrawal: On day 15, 24h after ethanol-withdrawal, experimental (ethanol diet) groups were treated with 6-Shogaol (10 and 30 mg/kg, p.o.) or fluoxetine (10 and 30 mg/kg, p.o.) or vehicle, and after 30 min, EPM activity of individual mouse was assessed. Values are expressed as mean±S.E.M (n= 6). Values are statistically significant at *p <0.001 vs. respective control group (One-way ANOVA followed by Bonferroni test).

Two-way ANOVA followed by Bonferroni test revealed that cronic treatment with 6-Shogaol (10 and 30 mg/kg, p.o.) to experimental (ethanol diet) group, significantly (p<0.05) increased the no. of entries in open arm evident at 6, 24, and 48h time interval after ethanol-withdrawal. Two-way ANOVA revealed a significant effect of 6-Shogaol treatment [F (3, 20) = 83.22, p< 0.0001] (Fig.-6). Fluoxetine (10 and 30 mg/kg, p.o.) had also a significant effect on EPM test [F (3, 20) = 96.58, p< 0.0001].

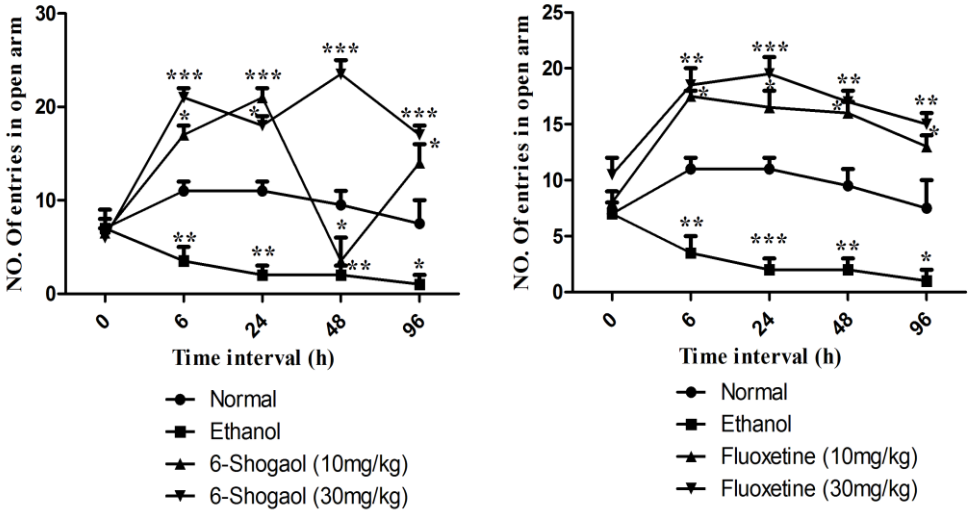

Fig. 6: Effect of chronic treatment with 6-Shogaol or fluoxetine on EPM test after ethanol-withdrawal: Experimental (ethanol diet) groups were treated with 6-Shogaol (10 and 30 mg/kg, p.o.) or fluoxetine (10 and 30 mg/kg, p.o.) or vehicle twice daily. Control group was daily treated with liquid diet (40 ml/day at 08:00 a.m.). On the 15th day, ethanol was withdrawn; EPM test of individual group of mouse was examined at 0, 6, 24, 48, and 96 h time intervals. Values are expressed as mean±S.E.M (n= 6). Values are statistically significant at *p <0.05 vs. respective control group, p<0.05 vs. respective vehicle treated experimental group (Two-way ANOVA followed by Bonferroni test).

V. DISCUSSION

The present study set out to investigate that, the effects of 6-Shogaol in ethanol-dependent mice using Fluoxetine as a control. Measures made in this model were consistent with literature data [52, 53] in that a daily ethanol consumption ranging from 24 to 30 g/kg yielding ethanol blood level close to 2 g/L (43 mM) produced the emergence of symptoms such as hyperexcitability and heightened anxiety due to ethanol treatment cessation in mice.

This report shows that ethanol-withdrawal on chronic administration decreases the no. of entries of mice in the light area, and acute treatment with 6-Shogaol dose dependently reverses their response. Chronic treatment with 6-Shogaol decreases the time expend in the light area on light & dark test and EPM test. In rodents, increased anxiety-like behavior

.

during withdrawal is likely a reflection of the direct effects of ethanol exposure on neuronal functioning affecting particularly the GABAergic transmission [54, 54]. It is known that the GABAergic system plays an important role in the control of anxiety, and dysfunction of GABA A receptors in some key brain structures might underlie anxious states. As indicated in the Introduction, the physical signs and increased anxiety during ethanol withdrawal might be attributable to differential alterations in GABAA receptors subunits function and expression [56]. The present study revealed that peak increase in light & dark test and EPM was observed at 24 h time interval after ethanol-withdrawal, which later declined to normal by 96 h. The ethanol - withdrawal state is characterized by serotonin dysfunction, and hyperactivity of dopamine and glutamate [57].

Further, it was observed that acute treatment with 6-Shogaol (10-30 mg/kg, p.o.), 30 min prior to the peak, dose dependently attenuated the increased light & dark test and EPM test in the ethanol - withdrawal state. The effect of 6-Shogaol was comparable to that of fluoxetine (10-30 mg/kg, p.o.). In addition, chronic treatment with 6-Shogaol (30 mg/kg) or fluoxetine (30 mg/kg), twice daily along with ethanol diet prevented an increase in light & dark test and EPM test evident after ethanol-withdrawal.

VI. CONCLUSION

The result of this study revealed the inhibitory influence of 6-Shogaol in ethanol withdrawal induced motivational effects, which may be due to modulatory action on various neurotransmitters and it caused anxiolytic effect in mice.

REFERENCES

[1.] Egli M., Crabbe M., Becker H. C., “Acute withdrawal protracted abstinence and negative effect in alcoholism: are they linked?,” Addict. Biol., Vol. 15, pp. 169–184, 2010.
[2.] Willinger A. B., Hoffman P.L., “Alcohol addiction: an enigma among us,” Neuronology, vol. 16, pp. 909-912, 1996.
[3.] Vengeliene V., Bilbao A. M., Spanagel R., “REVIEW Neuropharmacology of alcohol addiction.” British Journal of Pharmacology, vol. 154, pp. 299–315, 2008.
[4.] Hughes J.R., "Alcohol withdrawal seizures," Epilepsy Behav, vol. 15, no. 2, pp. 92–97, 2009. DOI:10.1016/j.yebeh.2009.02.037
[5.] Heilig U., Lenzinger E., Hornik K., Fischer G., Schonbeck G., Aschauer H. N., et al., “Anxiety as a predictor of relapse in detoxified alcohol-dependent patients,” Alcohol Alcohol, vol. 37, pp. 609–612, 2002.
[6.] De Witte P., Pinto E., Ansseau M., Verbanck P., “Alcohol and withdrawal: from animal research to clinical issues,” Neurosci. Biobehav. Rev., vol. 27, pp. 189–197, 2003.
[7.] Kliethermes C. L., “Anxiety-like behaviors following chronic ethanol exposure,” Neurosci. Biobehav. Rev., vol. 28, pp. 837–850, 2005.
[8.] Santucci A. C., Cortes C., Bettica A., Cortes F., “Chronic ethanol consumption in rats produces residual increases in anxiety 4 months after withdrawal,” Behav. Brain Res, vol. 188, pp. 24–31, 2008.
[9.] Martinotti G., Nicola M.D., Reina D., Andreoli S., Foca F., Cunniff A., Tonioni F., Bria P., Janiri L. "Alcohol protracted withdrawal syndrome: the role of anhedonia," Subst Use Misuse,vol. 43, no. 3–4, pp. 271–84, 2008.
[10.] Borras L., De Timary P., Constant E.L., Huguelet P., Eytan A., "Successful treatment of alcohol withdrawal with trazodone," Pharmacopsychiatry, vol. 39, no. 6, pp. 232, 2006.
[11.] Bayard M., McIntyre J., Hill K.R., Woodside J., "Alcohol withdrawal syndrome,"Am Fam Physician, vol. 69, no. 6, pp. 1443–1450, 2004. PMID 15053409
[12.] Amato L., Minozzi S., Vecchi S., Davoli M., Amato Laura ed., "Benzodiazepines for alcohol withdrawal," Cochrane Database Syst Rev, vol. 3, no. 3, 2010. CD005063
[13.] Ebell M. H., "Benzodiazepines for alcohol withdrawal," Am Fam Physician, vol. 73, no.7, pp.1191, 2006.
[14.] Toki S., Saito T., Nabeshima A., Hatta S., Watanabe M., Takahata N., "Changes in GABAA receptor function and cross-tolerance to ethanol in diazepam-dependent rats," Alcohol. Clin. Exp. Res., vol. 20, no. 1, pp. 40A–44A, 1996.
[15.] Ziegler P.P. "Alcohol use and anxiety," Am J Psychiatry, vol. 164, no. 8, pp. 1270 –127, 2007.
[16.] Ebadi M., "Alphabetical presentation of drugs," Desk Reference for Clinical Pharmacology, vol. 2, pp. 512, 2007.
[17.] Prince V., Turpin K.R., "Treatment of alcohol withdrawal syndrome with carbamazepine, gabapentin and nitrous oxide," Am J Health Syst Pharm, vol. 65, no. 11, pp. 1039–47, 2008.
[18.] Minozzi S., Amato L., Vecchi S., Davol M., Minozzi S., Minozzi S. ed., "Anticonvulsants for alcohol withdrawal," Cochrane Database Syst Rev, vol. 3, no. 3, pp. 2010.CD005064
[19.] Addolorato G., Leggio L., Abenavoli L., Agabio R., Caputo F., Capristo E., Colombo G., Gessa G. L., Gasbarrini G., "Baclofen in the treatment of alcohol withdrawal syndrome: a comparative study vs diazepam," Am J Med, vol. 119, no. 3, pp.13–8, 2006.
[20.] Kramp P., Rafaelsen O.J., "Delirium tremens: a double-blind comparison of diazepam and barbital treatment," Acta Psychiatr Scand, vol. 58, no. 2, pp. 174–90, 1978.
[21.] Baumgartner G.R., "Clonidine versus chlordiazepoxide in acute alcohol withdrawal: a preliminary report," South Med J, vol. 81, no. 1, pp. 56–60, 1988.
[22.] Dissanaike S., Halldorsson A., Frezza E.E., Griswold J., "An ethanol protocol to prevent alcohol withdrawal syndrome," J Am Coll Surg, vol. 203, no. 2, pp. 186–91, 2006.

.

[23.] Little H. J., "The benzodiazepines: anxiolytic and withdrawal effects," Neuropeptides, vol. 19, pp. 11–14, 1991.
[24.] Borras L., De Timary P., Constant E.L., Huguelet P., Eytan A., "Successful treatment of alcohol withdrawal with trazodone," Pharmacopsychiatry, vol. 39, no. 6, pp. 232, 2006.
[25.] Mihic S.J., "Acute effects of ethanol on $GABA_A$ and glycine receptor function," Neurochem. Int., vol. 35, pp.115-123,1999.
[26.] Kumar S., Fleming R.L., Morrow A.L., "Ethanol regulation of aminobutyric $acid_A$ receptors: Genomic and nongenomic mechanisms," Pharmacol. Ther., vol. 101, pp. 211-226, 2004.
[27.] Smith B.R., Horan J.T., Gaskin S., Amit Z., "Exposure to nicotine enhances acquisition of ethanol drinking by laboratory rats in a limited access paradigm," Psychopharmacology, vol. 142, pp. 408-412, 1999.
[28.] Carta M., Ariwodola, O.J., Weiner J.L., Valenzuela C.F., "Alcohol potently inhibits the kainite receptor-dependent excitatory drive of hippocampal interneurons," Proc. Natl. Acad. Sci., vol. 100, pp. 6813-6818, 2003.
[29.] Anders D.L., Blevins T., Sutton G. et al., "Fyn tyrosine kinase reduces the ethanol inhibition of recombinant NR1/NR2A but not NR1/NR2B NMDA receptors expressed in HEK 293 cells," J. Neurochem., vol. 72, pp. 1389-1393,1999.
[30.] Dopico A.M., Chu B., Lemos J.R., Treistman S.N., "Alcohol modulation of calcium-activated potassium channels," Neurochem. Int., 35, pp. 103-106, 1999.
[31.] Crabbe J.C., "Alcohol and genetics: New models," Am. J. Med. Genet., vol. 114, pp. 969-974, 2002.
[32.] Diamond I., Gordon A.S., "Cellular and molecular neuroscience of alcoholism," Physiol. Rev., vol. 77, pp. 1-20, 1997.
[33.] Chandler L.J., Harris R.A., Crews F.T., "Ethanol tolerance and synaptic plasticity," Trends Pharmacol. Science, vol. 19, pp. 491-495, 1998.
[34.] Crabbe J.C., "Alcohol and genetics: New models." Am. J. Med. Genet., vol. 114, pp. 969-974, 2002.
[35.] Li T.K., "Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking," J. Stud. Alcohol, vol. 61, pp. 5-12, 2000.
[36.] Lappalainen J., Long J.C., Eggert M., *et al.*, "Linkage of antisocial alcoholism to the serotonin $5\text{-}HT_{1B}$ receptor gene in two populations," Arch. Gen. Psychiatry, vol. 55, pp. 989-994, 1998.
[37.] Overstreet D.H., Knapp D.J., Breese G.R., Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors, Pharmacol Biochem Behav, vol. 78, pp. 459–464, 2004.
[38.] Kalivas P.W., Stewart J., "Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity," Brain Res Brain Res Rev, vol. 16, pp. 223- 244, 1991.
[39.] Tzschentke T.M., "Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues." Prog. Neurobiol, vol. 56, pp. 613–672, 1998.
[40.] Ali B., Blunden G., Tanira M., Nemmar A., "Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research," Food and Chemical Toxicology, vol. 46, no. 2, pp. 409-420, 2008.
[41.] Chaiyakunapruk N., Kitikannakorn N., Nathisuwan S., Leeprakobboon K., Leelasettagool C., The efficacy of ginger for the prevention of postoperative nausea and vomiting: a meta-analysis," Am. J. Obstet. Gynecol., vol. 194,pp. 95–99, 2006.
[42.] Huang Q., Iwamoto M., Aoki S., Tanaka N., Tajima K., Yamahara J., Takaishi Y., Yoshida M., Tomimatsu T., Tamai Y., "Anti-5-hydroxytryptamine 3 effect of galanolactone, diterpenoid isolated from ginger," Chem. Pharm. Bull., vol. 39, pp. 397–399, 1991.
[43.] Shim S., Kim S., Kwon Y.B., Kwon J., "Protection by [6]-shogaol against lipopolysaccharide-induced toxicity in murine astrocytes is related to production of brain-derived neurotrophic factor," Food and Chemical Toxicology, vol. 50, 597- 602, 2012.
[44.] Shim S., Kim S., Choi D.S., Kwon Y.B., Kwon J., "Anti-inflammatory effects of [6]-shogaol: potential roles of HDAC inhibition and HSP70 induction. Food Chem," Toxicology, vol. 49, pp. 2734–2740, 2011.
[45.] Kabuto H., Nishizawa M., Tada M., Higashio C., Shishibori T., Kohno M., "Zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone] prevents 6-hydroxydopamine induced dopamine depression in mouse striatum and increases superoxide scavenging activity in serum, Neurochem. Res, vol. 30, pp. 325–332, 2005.
[46.] Kaur G., Kulkarni S. K., "Investigations on possible serotonergic involvement in effects of OB-200G (polyherbal preparation) on food intake in female mice," Eur. J. Nutr., vol. 40, pp. 127-130, 2001.
[47.] Isaa Y., Miyakawa Y., Yanagisawa M., Goto T., Kang M.S., Kawada T., Morimitsu Y., Kubota K., Tsuda T., "6-Shogaol and 6-gingerol, the pungent of ginger, inhibit TNF-a mediated downregulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes," Biochemical and Biophysical Research Communications, vol. 373, pp. 429-434, 2008.
[48.] Sabina E.P., Rasool M.K., Mathew L., EzilRani P., Indu H., "6-Shogaol inhibits monosodium urate crystal-induced inflammation – An in vivo and in vitro study," Food and Chemical Toxicology, vol. 48, pp. 229–235, 2010.
[49.] Verleye M., Heulard I., Gillardin J.M., "The anxiolytic etifoxine protects against convulsant and anxiogenic aspects of the alcohol withdrawal syndrome in mice," Alcohol, vol. 43, 197-206. 2009.
[50.] Watson W.P., Little H. J., "Interactions between diltiazem and ethanol: differences from those seen with dihydropyridine calcium channel antagonists," Psychopharmacology (Berl), vol. 114, pp. 329–336, 1994.
[51.] Holmes A., Yang R.J., Crawley J.N., "Evaluation of an anxiety-related phenotype in galanin

.

overexpressing transgenic mice," J Mol Neurosci, vol. 18, pp. 151– 165, 2002.
[52.] Naassila M., Legrand E., d'Alche-Biree F., Daoust M., "Cyamemazine decreases ethanol intake in rats and convulsions during ethanol withdrawal syndrome in mice, Psychopharmacology (Berl), vol. 140, pp. 421–428, 1998.
[53.] Watson W. P., Little H. J., "Selectivity of the protective effects of dihydropyridine calcium channel antagonists against the ethanol withdrawal syndrome," Brain Res, vol. 930, pp. 111–122, 2002.
[54.] Liang J., Zhang N., Cagetti E., Houser C. R., Olsen R. W., Spigelman I.,Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal GABAA receptors, J. Neurosci, vol. 26, pp. 1749-1758, 2006.
[55.] Littleton J., "Neurochemical mechanisms underlying alcohol withdrawal," Alcohol Health Res. World, vol. 22, pp. 13-24, 1998.
[56.] Kumar S., Fleming R.L., Morrow A. L., "Ethanol regulation of gamma-aminobutyric acid A receptors: genomic and nongenomic mechanisms," Pharmacol. Ther., vol. 101, pp. 211-226, 2004.
[57.] Pierce R.C., Kumaresan V., "The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse?," Neurosci. Biobehav. Rev., vol. 30, pp. 215-238, 2006.