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Effect of 5-HT 1b/1d agonist on ethanol withdrawal syndrome and ethanol withdrawal induced anxiety

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Abstract

Introduction: *Ethanol dependence and abuse is an important problem of public health worldwide and its withdrawal shows some severe behavioural complication. Management of ethanol withdrawal syndrome (EWS) is still a challenge, thus the presented report postulates the possible mechanism involved in the development of EWS.*

Material and methods: *EWS was induced by administration of ethanol for 21 days and 5-hydroxytryptamine (5-HT) 1b/1d agonist treated group receives Zolmitriptan (ZMT) at 30 mg/kg i.p. 30 min prior to ethanol withdrawal. The effect of 5-HT 1b/1d receptor agonist on EWS was determined by estimating the change in the behaviour of withdrawal signs that included locomotor hyperactivity, agitation, tremor, tail stiffness, stereotyped behaviour, and wet dog shakes at 1, 2, 4, 6 and 12 h of ethanol withdrawal. Ethanol withdrawal induced anxiety was determined by using the elevated plus maze and levels of neurochemicals such as γ-aminobutyric acid (GABA), glutamate and dopamine were determined in the brain of each group of rats.*

Results: *Data of the given report reveal that Zolmitriptan reverses ($p < 0.01$) the behavioural changes induced due to EWS and also reduces the anxiety level in EWS rats. Moreover, Zolmitriptan was found to stimulate ($p < 0.01$) the level of GABA and ameliorate the level of other neurochemicals in the brain of EWS rats.*

Conclusions: *In conclusion, data of investigation reveal that 5-HT 1b/1d receptor involved in the EWS and treatment with its agonist prevents the behavioural changes in EWS by regulating the level of different neurochemicals.*

Key words: *ethanol withdrawal syndrome, 5-hydroxytryptamine 1b/1d receptor, Zolmitriptan, GABA.*

Introduction

Ethanol is widely consumed and abused worldwide. Dependence commonly occurs after chronic ethanol administration and its withdrawal is associated with ethanol withdrawal syndrome (EWS). Development of physical dependence is the most common evidence of EWS [8]. Management of EWS has limited choices, as disulfiram, naltrexone and acamprosate are the few approved drugs for it [10]. However, these drugs are not treating EWS but reduce only consumption and cravings of ethanol. Overlapping clinical features was observed between addictive disorders and schizophrenia. The study suggests that chances of risk of alcohol abuse and addiction observed are higher in schizophrenia patients [3,15] and an alcoholic has four times more incidences of schizophrenia than a non-alcoholic [11]. Moreover drug/alcohol dependence is accepted widely as a prognostic factor for psychosis [16].

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Alcohol dependence is reported to alter the neurotransmission including serotonergic [9]. An alcoholic commonly shows the symptoms of craving and impulsivity and serotonin has been experimentally proven to have these effects [5], it is also involved in dependence of alcohol [13,17]. Evidence suggests that in the reward area of brain, alcohol induced firing of dopaminergic neuron potentiated due to serotonin, which contributes to the development of alcohol addiction by promoting its reinforced effect [19]. There are several serotonergic receptors available in the central nervous system (CNS) including 5-hydroxytryptamine (5-HT) 1b/1d receptor. These receptors are present on the membrane of the presynaptic neuron, act as an auto receptor, inhibit the postsynaptic excitatory potential by regulating the release of glutamate [1]. Moreover, it also controls the release of GABA and dopamine, too. Zolmitriptan is an antimigraine drug, which is an agonist of this receptor. It is also reported to show the analgesic effect and protective effect against cocaine disorder. Thus, this report determines the effect of Zolmitriptan against EWS.

Material and methods

Animal

Adult male Wistar rats (200-250 g) were housed under controlled conditions (temperature of 24 ±3°C and 60 ±5% humidity) with a 12 h light/dark cycle and used in this study. Exposure to ethanol and all behavioural experiments involved in EWS were carried out in different separate and isolated laboratories, which have the same environmental conditions as the colony room. All the animal experimentation was approved by the Institutional Animal Ethics Committee of P. Wadhwani College of Pharmacy, Yavatmal (650/02/C/CPCSEA/01).

Chemicals

Zolmitriptan was supplied by Zim Laboratories, India. Glutamate, GABA, and dopamine assay kits were procured from Abcam Ltd., USA. Ethanol was purchased from SD Fine Chemical Ltd., India.

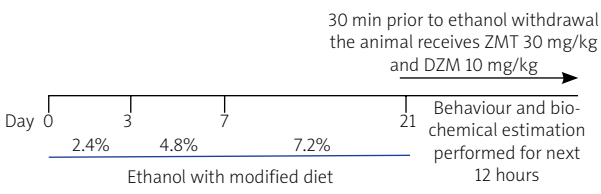

Fig. 1. Diagrammatic representation of the experimental timeline.

Experiments

A pilot study was performed for the assessment of the effect of Zolmitriptan at 17 mg/kg, 25 mg/kg and 30 mg/kg i.p. on EWS and it was shown that Zolmitriptan at 30 mg/kg i.p. effectively ameliorates the altered behavioural parameters in EWS rats. Further a study was performed on Zolmitriptan at a dose of 30 mg/kg i.p.

Chronic ethanol exposure

All the rats were housed individually, and ethanol was given in the modified liquid diet. No extra chow or water was supplied to the rats. The composition of the modified liquid diet with ethanol was: cow milk 925 ml, 25-75 ml ethanol (96.5% ethyl alcohol), vitamin A 5000 IU and sucrose 17 g [21]. All the rats were given modified liquid diet without ethanol for 7 days for the habituation. Then, liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 4 days and finally to 7.2% for 14 days. Liquid diet was freshly prepared daily and presented at the same time of the day (09:30 h). The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g per kg per day. Control rats (n = 8) were pair fed with an isocaloric liquid diet containing sucrose as a caloric substitute to ethanol.

Evaluation of ethanol withdrawal syndrome (EWS)

Ethanol was withdrawn and replaced with isocaloric ethanol-free diet at 09:30 h at the end of 14th day of protocol. Ethanol-dependent rats were then assigned into four groups randomly (n = 8 for each group). The control group received ethanol-free modified diet; the negative control group received ethanol with modified diet; and the ZMT group received ethanol with modified diet and 30 min prior to ethanol withdrawal animals received Zolmitriptan 30 mg/kg, i.p.; the DZM group received ethanol with modified diet and 30 min prior to ethanol withdrawal animals received diazepam (DZM) 10 mg/kg i.p. Ethanol withdrawal symptoms such as locomotor hyperactivity, agitation, tremor, tail stiffness, stereotyped behaviour and wet dog shakes were assessed by placing the rat in an open field apparatus at 1, 2, 4, 6 and 12 h of ethanol withdrawal (Fig. 1).

Evaluation of anxiety by the elevated plus maze

The elevated plus maze was used to determine the level of anxiety in the rodent model as per the reported method. The elevated plus maze consists of two open and two enclosed arms, each with an open roof, elevated 40-70 cm from the floor. The model is based on rodents' aversion of open spaces. In EPM this translates to a restriction of movement to the enclosed arms. Anxiety reduction in the plus maze was indicated by increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). The total number of arm entries and number of closed-arm entries were usually employed as measures of general activity or anxiety.

Evaluation of locomotor activity

Locomotor behaviour was assessed by recording each animal for 5 min using actophotometer. Locomotor activity was recored digitally, as rats block the beam of light falling on photocell while doing locomotion.

Preparation of brain tissue homogenate

All the animals were sacrificed using cervical dislocation and isolated brain tissue was homogenized in 0.1 M phosphate buffer (pH = 7.4). Brain tissue homogenate was centrifuged for the period of 15 min at 3000 rpm and supernatant was used for the estimation of the biochemical.

Estimation of dopamine, glutamate, and GABA

The level of glutamate, γ-aminobutyric acid (GABA), and dopamine in the brain tissue homogenate were assessed using their respective assay kits as per the instructions given by the manufacturer of kits.

Statistical analysis

Results were expressed as mean ±SEM (n = 8). The statistical analysis was performed using one way ANOVA followed by Dunnett test for multiple comparisons (GraphPad Prism software, ver. 6.1; USA). The level of statistical significance was set at $p < 0.05$.

Results

Effect of 5-HT 1b/1d agonist on body weight and ethanol consumption

Effect of 5-HT 1b/1d agonist was observed on body weight and ethanol consumption in the experimental ethanol withdrawal syndrome rat model as shown in Figure 2A, B. There was an increase in body weight of the control group of rats up to 18.22% from 0th day of the protocol to 21st day of the protocol of ethanol exposure. The negative control group shows an increase in body weight up to 2.6% from

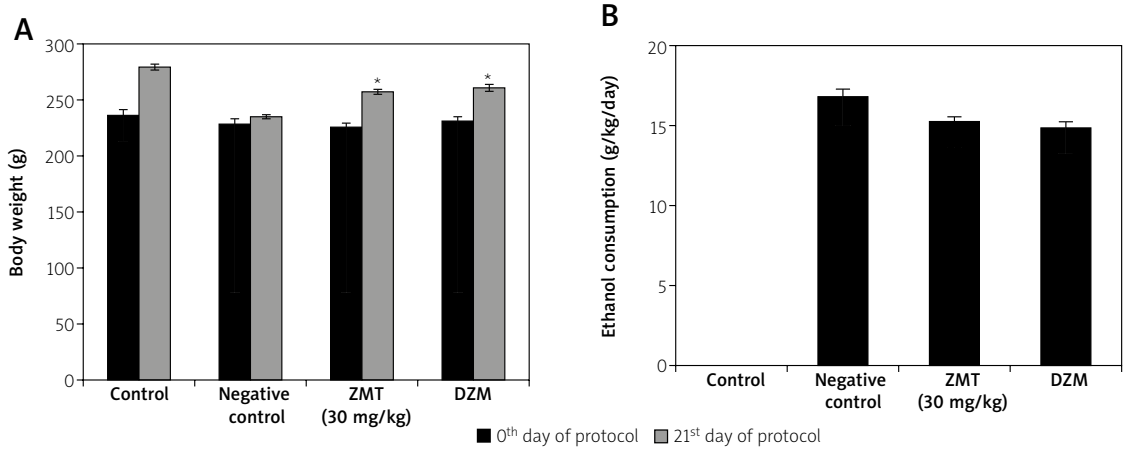

Fig. 2. Effect of 5-HT 1b/1d agonist on the body weight and ethanol consumption in experimental ethanol withdrawal syndrome rats. **A**) Effect of 5-HT 1b/1d agonist on change in body weight on 0th and 21st day of the ethanol exposure protocol; **B**) Effect of 5-HT 1b/1d agonist on ethanol consumption in 7.2% of ethanol exposure rats. Data were expressed as mean ±SEM (n = 8); *$p < 0.01$ compared to 0th day of the protocol.

0th day of the protocol to 21st day of the protocol of ethanol exposure. Treatment with ZMT and DZM shows an increase in body weight up to 14.2% and 12.9% from 0th day of the protocol to 21st day of the protocol of ethanol exposure (Fig. 1A). Ethanol consumption was observed in the ethanol exposed group as shown in Figure 1B. Ethanol consumption was observed in the negative control, ZMT and DZM group as 16.9, 15.3 and 14.9 g/kg on 7.2% v/v of ethanol exposed rats.

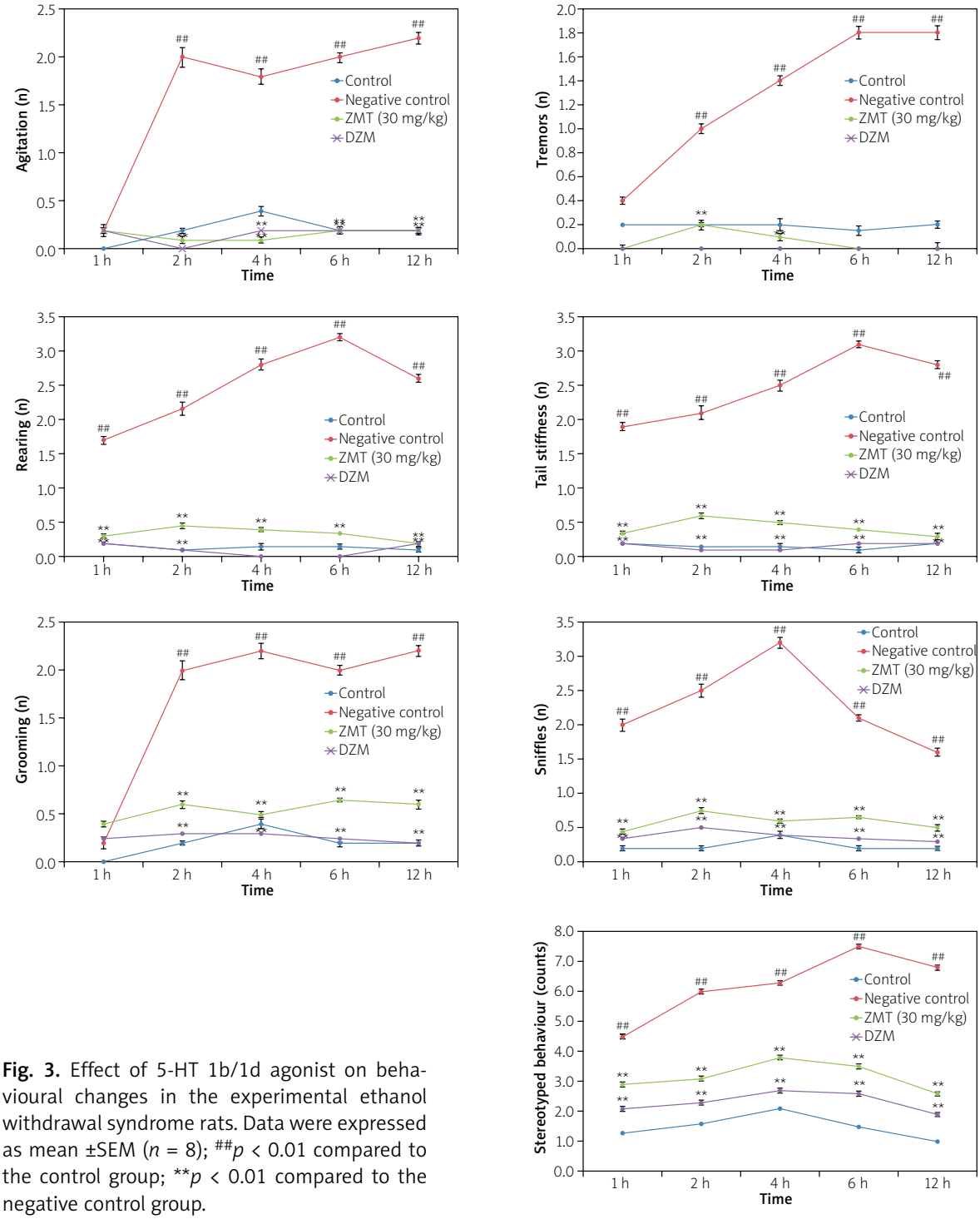

Fig. 3. Effect of 5-HT 1b/1d agonist on behavioural changes in the experimental ethanol withdrawal syndrome rats. Data were expressed as mean ±SEM (n = 8); $^{\#\#}p < 0.01$ compared to the control group; $^{**}p < 0.01$ compared to the negative control group.

Effect of 5-HT 1b/1d agonist on the altered behaviour of ethanol withdrawal syndrome rats

Behavioural changes such as agitation, tremor, rearing, tail stiffness, grooming, sniffles, and stereotyped behaviour were observed in 5-HT 1b/1d agonist treated ethanol withdrawal syndrome model rats as shown in Figure 3. There was a significant increase ($p < 0.01$) in the number of agitation, tremor, rearing, tail stiffness, grooming, sniffles and stereotyped behaviour changes at 1, 2, 4, 6 and 12 h of ethanol withdrawal in the negative control group compared to the control group of rats. However, treatment with ZMT and DZM ameliorates the altered behavioural changes in EWS rats.

Effect of 5-HT 1b/1d agonist on locomotor activity

Locomotor activity was assessed in 5-HT 1b/1d agonist treated EWS rats using am actophotometer as shown in Figure 4. There was a significant increase ($p < 0.01$) in locomotor activity in the negative control group compared to the control group of rats. There was a significant reduction ($p < 0.01$) in locomotor activity in ZMT and DZM treated groups compared to the negative control group rats.

Effect of 5-HT 1b/1d agonist on the level of anxiety

The elevated plus maze was used to estimate the level of anxiety in 5-HT 1b/1d agonist treated EWS rats as shown in Table I. Percentage of open arm and close arm entries was determined in the elevated plus maze, there was a significant decrease ($p < 0.01$) in open arm entries and increase in close arm entries in the negative control group compared to the control group of rats. There was a significant increase in percentage of open arm entries and reduction in close arm entries in ZMT and DZM treated groups compared to the negative control group of rats.

Table I. Effect of Zolmitriptan on ethanol withdrawal syndrome induced anxiety using the elevated plus maze

No.	Group	Open arm entry (%)	Close arm entry (%)
1	Control	39.62 ±2.76	60.38 ±3.12
2	Negative control	12.65 ±1.29##	87.35 ±3.84##
3	ZMT (30 mg/kg)	28.47 ±1.94**	71.53 ±2.79**
4	DZM	32.74 ±1.57**	67.26 ±2.33**

*Data were expressed as mean ±SEM (n = 8); ##p < 0.01 compared to the control group; **p < 0.01 compared to the negative control group.*

Effect of 5-HT 1b/1d agonist on the level of neurotransmitters

The levels of neurochemicals such as dopamine, GABA and glutamate were estimated in brain tissue of 5-HT 1b/1d agonist treated ethanol withdrawal rats as shown in Figure 5. There was a reduction in the level of GABA and dopamine and an increase in the level of glutamate in the brain tissue of the negative control group compared to the control group of rats. However, treatment with ZMT and DZM ameliorates the altered

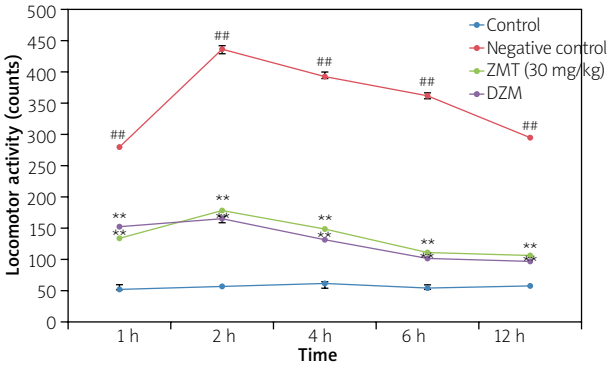

Fig. 4. Effect of 5-HT 1b/1d agonist on locomotor activity in experimental ethanol withdrawal syndrome rats. Data were expressed as mean ±SEM ($n = 8$); ##$p < 0.01$ compared to the control group; **$p < 0.01$ compared to the negative control group.

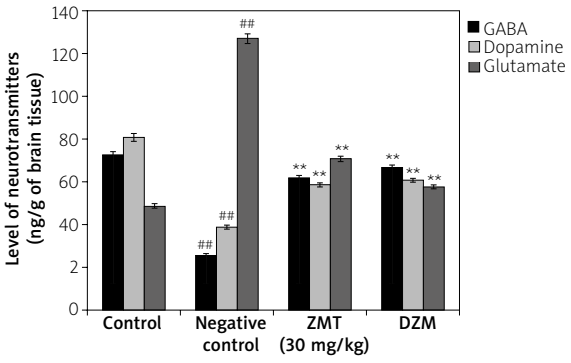

Fig. 5. Effect of 5-HT 1b/1d agonist on the level of dopamine, GABA and glutamate in the brain tissue of ethanol withdrawal rats. Data were expressed as mean ±SEM ($n = 8$); ##$p < 0.01$ compared to the control group; **$p < 0.01$ compared to the negative control group.

levels of dopamine, GABA, and glutamate in brain tissue of EWS rats.

Discussion

Ethanol abuse and its habituation is one of the major public issues throughout the globe. Consumption of ethanol for the duration of 21 consecutive days causes physical dependence and its withdrawal causes several behavioural changes such as agitation, tail stiffness, sniffles, tremors, rearing, grooming and stereotyped behaviour [4]. The literature reveals that administration of ethanol at 9-15 g/kg/day for more than four consecutive days causes dependence and its withdrawal alters the behavioural signs [6]. Data of present report level of ethanol exposure reached to 14.9-16.9 g/kg/day in ethanol administered groups. Behavior was observed to be changed in negative control group compared to control group. Moreover, treatment with 5-HT 1b/1d receptor agonist ameliorates the altered behavioural changes in EWS rats.

Dependence on ethanol consumption occurs due to the euphoric effect provided by dopamine, involvement of neurotransmitters affects behaviour and thus its withdrawal contributes to alteration of several behaviours such as agitation, tail stiffness, sniffles, tremors, rearing, grooming and stereotyped behaviour [18]. Data of the investigation reveal similar changes in behaviour of EWS rats. Drugs used to manage EWS majorly contribute to the amelioration of these altered behaviours in rats and results support that treatment with 5-HT 1b/1d agonist i.e. ZMT ameliorates the altered behavioural changes in EWS rats.

Evidence suggests serotonin and dopamine are majorly involved in ethanol dependence [14]. There is a strong relationship observed between ethanol withdrawal and development of anxiety. There are several anti-anxiety drugs such as diazepam which show potential to reduce anxiety associated with EWS and effectively manage EWS [23]. 5-HT 1b/1d receptor is an auto receptor, which regulates/controls the release of several neurotransmitters including GABA and Zolmitriptan is an agonist of 5-HT 1b/1d receptor as it enhances the release of GABA [20]. Modulation of GABA contributes to anti-anxiety activity and also relaxes the altered behaviour due to ethanol withdrawal. Data of the study suggest that treatment with 5-HT 1b/1d agonist reduces anxiety in EWS rats.

5-HT 1b/1d receptor is available on the preganglionic nerve and regulates the release of several neurochemicals in the brain. Zolmitriptan, which is a 5-HT 1b/1d receptor agonist acts as an anti-migraine, analgesic drug, neuropathic pain killer, and a depressant [12]. The dopamine level enhances in the brain due to ethanol intake and chronic exposure of ethanol reduces sensitivity of the brain cells to feel euphoric and to achieve a required high amount of ethanol, which develops dependence [2]. Moreover GABA and glutamate are the neurotransmitters which contribute to the regulation of normal behaviour of an individual as GABA depresses brain activity and glutamate stimulates it [22]. In EWS the level of these neurochemicals in the brain tissue gets disturbed, which leads to change in the behaviour and data of the study also support this. However, Zolmitriptan increases the level of GABA and reduces the level of glutamate in brain tissue of EWS rats and thereby ameliorates the altered behaviour of ethanol withdrawal rats [23]. Moreover, the level of dopamine also attenuates in the brain tissue of EWS rats, which contributes to the development of EWS.

Conclusions

In conclusion, data of the report reveal the beneficial effect of 5-HT 1b/1d receptor agonist against ethanol withdrawal syndrome and anxiety associated with it by regulating the level of neurochemicals viz. GABA, dopamine and glutamate in the brain tissue. The results suggest the potential role of Zolmitriptan in the management of EWS and it could be used for further investigation.

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Disclosure

The authors report no conflict of interest.

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