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# Protective Effect of Bupropion on Alcohol Abstinence-Induced Anxiety and Convulsions

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**Chronic administration of ethanol (2g/kg po) on days 1-6 and its withdrawal produced anxiogenic reaction in mice as assessed in the mirror chamber test. Daily administration of bupropion (2 or 5 mg/kg, ip) prior to ethanol for 6 days prevented withdrawal-induced anxiety in mice. However, acute administration of a single dose of bupropion (5 mg/kg), to animals withdrawn from ethanol, i.e. on the 7th day, did not prevent withdrawal-induced anxiety. Ethanol withdrawal also induced a significant increase in the locomotor activity of mice indicating an anxiogenic response. Administration of bupropion (2 or 5 mg/kg) prior to ethanol for 6 days also prevented withdrawal induced increased locomotor activity. Ethanol withdrawal also sensitized the convulsogenic reaction to pentylenetetrazole. A non convulsive dose (40-60 mg/kg) of pentylenetetrazole produced full blown convulsions and increased mortality rate in ethanol withdrawn mice. Both acute and chronic administration of bupropion (2 and 5 mg/kg) exhibited a significant protection against ethanol withdrawal-induced reduction in pentylenetetrazole threshold in mice.**

Physical dependence on ethanol is defined by the presence of an ethanol withdrawal syndrome that becomes apparent following cessation of ethanol intake and elimination of ethanol from the system. Tolerance to the effects of ethanol can be demonstrated in both humans and experimental animals but the mechanism of this tolerance is not known for certain[1]. The withdrawal symptom is well characterized in humans and in animal models[2,3], and it consisted of anxiety response, increased locomotion and convulsions (reduction in convulsive threshold) in animals. Alcoholism and withdrawal of its chronic intake are grave social and medical problems. There is no single drug therapy that helps patients in overcoming alcoholism[4].

Bupropion HCl, an antidepressant of the aminoketone class, is chemically unrelated to classical or second generation selective serotonin re-uptake inhibitor, or other known antidepressant agents. Bupropion is a noradrenaline and dopamine re-uptake inhibitors (NDRI) which has both noradrenergic and dopaminergic activity[5,6]. Bupropion has been recently licensed for the treatment of smoking cessation in a number of countries[7-11].

The present study was undertaken to evaluate the de-addiction potential of bupropion against alcohol withdrawal reactions in animals. The parameters used were alcohol withdrawal-induced anxiety, locomotion and reduction in convulsive threshold to PTZ in mice.

## MATERIALS AND METHODS

Laka mice of either sex (20-25 g), bred in Central Animal House facility of the Panjab University, Chandigarh, were housed 5 per cage at room temperature and allowed to adapt to laboratory conditions for at least 2 days before the initiation of any experiment. The animals were housed under on a natural light and dark cycle, and had free access to food and water. Each animal was used only once. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The drugs used were bupropion (Zyban®; Glaxo-

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SmithKline, UK), pentylenetetrazol (PTZ, Sigma, USA) and ethanol (Bengal Chemicals & Pharmaceuticals Ltd, Kolkata).

PTZ and ethanol were prepared in distilled water. Bupropion powder was dissolved in distilled water and administered i.p. Ethanol (2 g/kg of 10% v/v in mice) was administered orally while PTZ (40–80 mg/kg) was injected i.p. PTZ was administered 24h after the last dose of ethanol (withdrawal animals) and bupropion was given 30 min prior to ethanol.

**Treatment schedule:**

In chronic studies, mice received (2g/kg of 10 % v/v) ethanol, intragastrically, twice a day on 1st day and once daily on the successive days for a total of 6 days. On 7th day, i.e. 24 h after the last dose of ethanol mice were tested for withdrawal reaction. The other treatment groups included (pretreatment:treatment) : (i) saline:saline (ii) saline:bupropion (2 or 5 mg/kg; i.p.) (iii) saline:ethanol, and (iv) bupropion (2 or 5 mg/kg; ip):ethanol.

Control experiments were performed on day 7 to determine whether bupropion prevented the development of withdrawal syndrome or it simply altered the behavioral expression of the withdrawal symptoms. On this day, the treatments were reversed so that the animals that had received bupropion followed, 30 min. later, by ethanol on days 1 through 6 were challenged with saline. Similarly, the animals which received saline followed, 30 min later, by ethanol received only bupropion. The group chronically treated with saline received the same (saline only). The withdrawal reaction was assessed by studying the anxiogenic reaction in mirror-chamber and for decrease in threshold to pentylenetetrazol (proconvulsive response to an otherwise non-convulsive dose in naïve-animals).

**Measurement of anxiety and locomotor activity:**

The mirror chamber used for mice consists of a wooden chamber having a mirror-chamber enclosed within it. During the 5 min test session the following parameters were noted: (i) transfer latency, (ii) the total time spent in mirror-chamber, (iii) number of entries the animal made in mirror-chamber. Animals were put individually at the distal corner of the mirror chamber facing towards the mirror-chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber[12]. The ethanol withdrawal-induced hyperlocomotor activity of mice was measured in actophotometer for a period of 5 min.

**PTZ-threshold:**

The onset of body jerks, clonic convulsions followed by tonic convulsions and death were recorded following PTZ challenge in naïve and ethanol withdrawn animals. Each animal was observed individually for 2 h for acute response. Reduction in the dose of PTZ to produce full blown convulsions in ethanol withdrawn animals was considered as withdrawal-induced reduction in the convulsive threshold[13]. The protective effect of bupropion following acute and chronic treatment was noted.

**Statistical analysis:**

The data expressed as mean±SEM were analysed by Student' t-test. Probability levels < 5% were considered significant.

## RESULTS

**Effect of bupropion on ethanol withdrawal-induced hyperlocomotor activity in mice:**

Mice withdrawn from chronic ethanol treatment showed hyperlocomotor activity as compared with the vehicle-treated control group (fig. 1). Concomitant administration of bupropion (2 or 5 mg/kg, i.p.) along with ethanol showed reduced withdrawal-induced hyperlocomotor activity (fig.1).

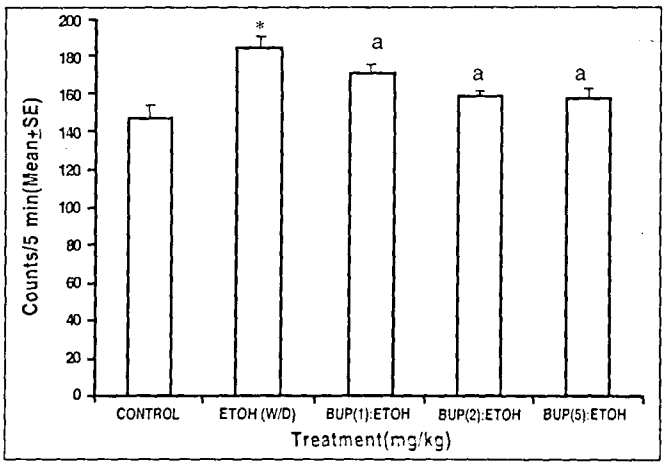


**Fig. 1: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the locomotor activity of mice.**

**Effect of co-administration of bupropion (BUP) on the long-term ethanol (2 g/kg) chronic treatment- and withdrawal-induced changes in the locomotor activity in mice. Values expressed mean±SEM (5–8 mice). *P<0.05 as compared to control group. aP<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Turkey's test).**

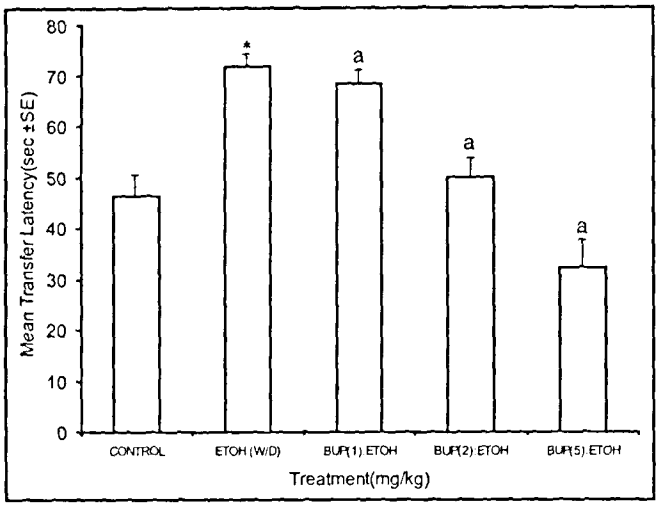


**Fig. 2: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the transfer latency to the mirror chamber in mice.**

**Effect of co-administration of bupropion (BUP) on the long-term ethanol (2 g/kg) chronic treatment- and withdrawal-induced changes in the transfer latency to the mirror chamber in mice. Values expressed mean±SEM (5–8 mice). *P<0.05 as compared to control group. #P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey's test).**

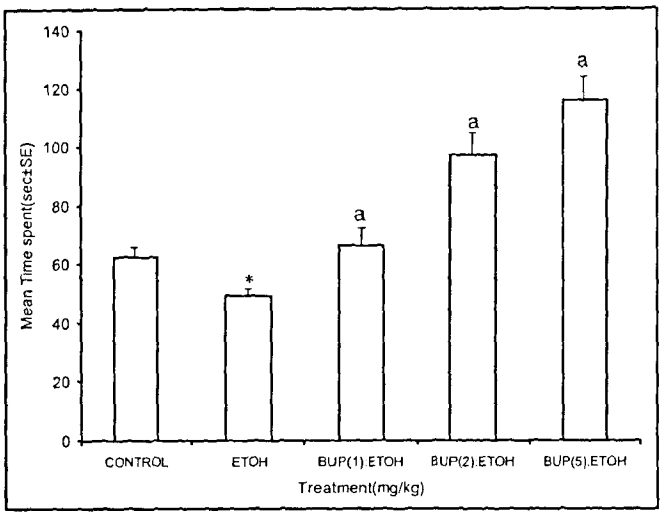


**Fig. 3: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the mean time spent in the mirror chamber in mice.**

**Effect of co-administration of bupropion (BUP) on the long-term ethanol (2 g/kg) chronic treatment- and withdrawal-induced changes in the mean time spent in the mirrored chamber in mice. Values expressed mean±SEM (5-8 mice). *P<0.05 as compared to control group. #P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey's test).**

Mice treated repeatedly with saline followed by ethanol for 6 days and then challenged with bupropion on 7th day did not show a significant decrease in the ethanol withdrawal induced hyperlocomotor activity (fig. 1).

**Effect of bupropion on ethanol withdrawal-induced anxiety:**

Mice withdrawn from chronic ethanol treatment showed lower preference for mirror-chamber, increase in transfer latency to the mirror chamber and significant decrease in the duration of time spent and number of entries in mirror-chamber as compared with the vehicle-treated control group.

Concomitant administration of bupropion (2 or 5 mg/kg, ip) along with ethanol chronically for 6 days showed reduced withdrawal-induced anxiety. There was a significant decrease in the transfer latency to the mirror-chamber (fig. 2). The time spent (fig. 3) and the number of entries (fig. 4) in mirror-chamber increased significantly as compared with ethanol-withdrawn group.

Mice treated repeatedly with saline followed by ethanol for 6 days and then challenged with bupropion on 7th day did not show reduced withdrawal-induced anxiety.

**Effect of bupropion on PTZ-threshold in ethanol-withdrawn animals:**

PTZ (80 mg/kg; i.p.) induced severe clonic-tonic seizures followed by 100 % mortality in naïve mice. A lower dose (60 mg/kg; i.p.) induced mild clonic-tonic seizures with reduced mortality, whereas a still lower dose (40 mg/kg; i.p.) failed to cause any mortality or observable behavioral change in naïve mice. However, in ethanol-withdrawn mice PTZ (60 mg/kg; i.p.) produced severe clonic-tonic seizures and 100 % mortality. Acute administration of bupropion (2 or 5 mg/kg; i.p.) to ethanol withdrawn mice showed protection against PTZ (60 mg/kg; i.p.) convulsions. Animal showed only mild clonic convulsions followed by recovery. Chronic administration of bupropion (2, 5 mg/kg; i.p.) followed by ethanol for 6 days exhibited only mild clonic seizures with delayed onset and 60% mortality following administration of PTZ (60 mg/kg; i.p.) on day 7 in mice (fig. 5).

## DISCUSSION

Chronic ethanol administration is known to produce

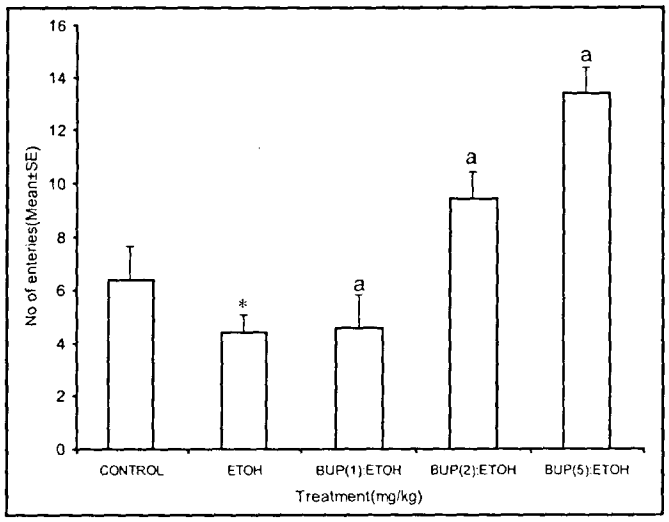


**Fig. 4: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the mean number of entries to the mirror chamber by mice.**

**Effect of co-administration of bupropion (BUP) on the long-term ethanol (2g/kg) chronic treatment and withdrawal-induced changes in the mean number of entries in the mirrored chamber in mice. Values expressed mean±SEM (5-8 mice). *P<0.05 as compared to control group. [a]P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey's test).**

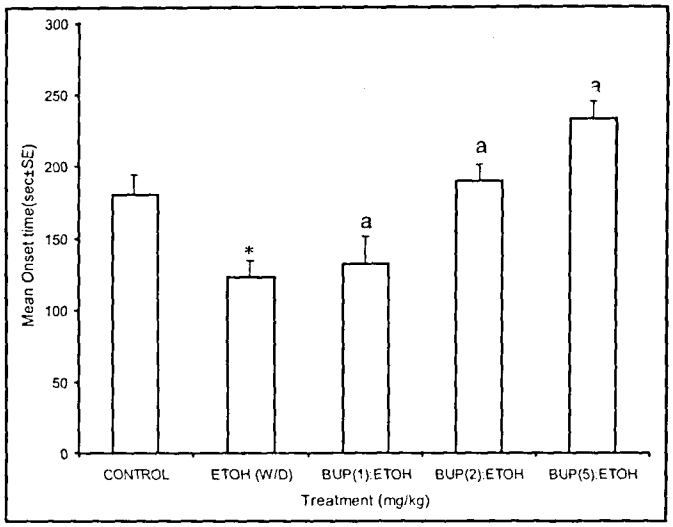


**Fig. 5: Effect of bupropion (BUP) on mean onset time after PTZ challenge in ethanol withdrawn mice.**

**Effect of co-administration of bupropion (BUP) on mean onset time after PTZ challenge (measurement convulsive threshold) in ethanol (ETOH)-withdrawn mice. Values expressed mean±SEM. [a]P<0.05 as compared to vehicle treated group. *P<0.05 as compared to control group. [a]P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey's test).**

behavioral and biochemical changes in humans and animals. Withdrawal from chronic intake of ethanol is also known to produce withdrawal reactions which range from craving behavior to severe seizures[3,14]. Although the exact cellular and biochemical mechanism of development of tolerance and dependence to ethanol is not fully understood, recent studies have, however, focused attention to the involvement of inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the action of ethanol[15]. At pharmacologically active concentrations ethanol produces some of its pharmacological effects through GABAergic transmission[16,17]. In the present study, acute administration of ethanol produced a significant increase in the time spent in mirror-chamber, thereby suggesting an anxiolytic profile of ethanol in mice. Biochemical studies have provided evidence that enhancement of GABA-mediated neuronal chloride fluxes may contribute to the sedative and anxiolytic profile of ethanol[17-19]. The observed anxiogenic action following ethanol withdrawal would, therefore, be consistent with the notion that GABAergic system is down-regulated during withdrawal. The animals treated chronically with bupropion followed by ethanol for 6 days displayed significant reversal of withdrawal-induced anxiety on 7th day. Acute administration of bupropion on 7th day to ethanol-withdrawn animals also displayed considerable anxiety on 7th day. The anxiety reaction in ethanol-withdrawn animals may be attributed to the anxiogenic action of bupropion per se or to an acute interaction between bupropion and ethanol. Reversal of anxiogenic response and hyperlocomotor activity following substitution of bupropion with saline in ethanol-withdrawn animals suggests that bupropion need not be present during testing to observe anxiety in chronically bupropion treated animals.

PTZ, a chemoconvulsant, is reported to induce seizures by depressing chloride channel function by binding to picrotoxin site on the GABA receptor complex[13]. In ethanol withdrawn animals lower doses of PTZ as compared to control animals was required so as to exhibit severe tonic seizures and mortality. Sensitization of inverse agonistic site on GABA-benzodiazepine receptor following ethanol withdrawal has been shown to be responsible for decrease in convulsive threshold to PTZ in ethanol-withdrawn animals[20]. Bupropion significantly inhibited PTZ convulsions

in these animals when given repeatedly for 6 days. Acute bupropion administration following ethanol withdrawal also produced significant attenuation of PTZ convulsions. The reversal of ethanol withdrawal sensitized convulsant response to PTZ suggests the effectiveness of this drug in suppressing ethanol withdrawal syndrome whether given acutely or chronically. The above data suggests that the anticonvulsant effect of bupropion alone or its acute interaction with ethanol is unlikely to account for its protective effect in addiction.

Bupropion is a safe drug having no apparent response *per se* on reward system. Concurrent administration of this drug may help in preventing the development of tolerance and dependence to ethanol and other psychotropic drugs. The drug is also effective in combating withdrawal reactions to chronic administration of ethanol.

Bupropion and its active metabolites compete for the neuronal transporters for dopamine (DA) and norepinephrine (NE), and thus, increase extracellular levels of these monoamines in synapses within the brain and in the interstitial spaces between the cells. Bupropion blocks reuptake of NE and DA, and also blocks nicotinic receptors in the low to intermediate micromolar range [21-22]. The antidepressant effects of bupropion result from inhibition of dopamine and norepinephrine transporters (DAT and NET, respectively) however, its mechanism of action is not fully understood[23]. Most drugs of dependence preferentially stimulate the release of DA in the nucleus accumbens, a principal terminal field of the mesolimbic system, and this property is fundamental to the addictive potential of all drugs of abuse[24]. Bupropion-induced inhibition of DAT and NET function and associated increases in extracellular DA and NE concentrations, respectively, may substitute for drug-evoked neurotransmitter release[25].

Bupropion is a selective re-uptake inhibitor of dopamine and noradrenaline which prevents or reduces cravings and other features of nicotine withdrawal. The effects of bupropion on addiction may be through dual effects on dopaminergic and nicotinic systems. Its effects on DA, NE and indirectly 5-HT overflow in the brain may also contribute to attenuation of other symptoms associated with abstinence[21,26]. Bupropion sustained release preparation provided the first non-nicotine pharmacological treatment approved for smoking cessation and was thought to be effective because of its dopaminergic activity on the pleasure and reward pathways in the mesolimbic system and nucleus accumbens[27]. The underlying mechanism for bupropion in smoking cessation is thought to be mediated by increasing the concentration of dopamine in the nucleus accumbens[28]. Thus, bupropion is believed to work by elimination of nicotine cravings and to decrease the physiologic and psychologic symptoms associated with nicotine withdrawal[29].

There are many evidences of interaction of bupropion with dopaminergic system. Certain *in vivo* studies indicated that bupropion is a selective dopamine uptake inhibitor and that dopaminergic system plays an important role in its central nervous system pharmacology.

Bupropion is known to induce seizures in high doses *per se*[30-32]. Recently, Tutka *et al.* (2004) demonstrated that bupropion hydrochloride a commonly used smoking cessation aid, dose-dependently caused clonic convulsions in mice, with the CD50 (convulsive dose 50, i.e., the dose producing convulsions in 50% of mice) at 119.7 mg/kg. An evaluation for anticonvulsant effects showed that bupropion in the doses of 15-30 mg/kg protected against convulsions induced by maximal electroshock with the ED50 (effective dose50, i.e., the dose protected 50% of mice against convulsions) being 19.4 mg/kg. Bupropion had no effect on pentylenetetrazole- and kainic acid-induced convulsions. It was speculated that the anticonvulsant activity of bupropion may be exploited for use in the treatment of epilepsy but it requires further investigations[33].

Thus the present study provides evidence for the de-addiction potential of acute as well as chronic administration of bupropion against ethanol. The observed effect may be due its interaction with dopamine in reward areas or any other neurotransmitter effecting dependence, but the exact mechanism remains to be explored.

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