doi.org/10.36721/PJPS.2024.37.5.REG.1063-1069.1

Effects of bupropion on nicotine withdrawal associated disturbances in circulating corticosterone and brain 5-HT turnover in mice

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Abstract: Bupropion (Bup), an antidepressant, is used to treat depression and aid in quitting smoking. We aim to investigate the influence of Bup on nicotine withdrawal (NW)-associated disturbances in serotonergic neurotransmission and behavior in mice. Adult albino mice were categorized into control and NW groups. Each group was further divided into saline and Bup-administered (n=6/group). NW groups received nicotine at a concentration of 3.08 mg (equivalent to 1 milligram of free base) in 100 ml of tap water for four weeks, while the control group received nicotine-free water. To induce nicotine withdrawal, the nicotine-containing water was substituted with tap water for 72 hours. Bup (20 mg/kg) and saline were administered (i.p.) three hours before the completion of the 72-hour withdrawal period to the test and control groups, respectively. NW signs were monitored in both groups. Bup-treated NW mice demonstrated a decline in corticosterone levels while concurrently exhibiting an increase in 5-HT synthesis with decreased 5-HT turnover compared to NW saline controls. A positive correlation between plasma corticosterone and 5-HT turnover was also found in Bup-administered NW mice. Taken together, Bup has potential therapeutic effects on nicotine withdrawal-associated somatic signs due to its ability to attenuate 5-HT turnover and plasma corticosterone in dependent mice.

Keywords: Nicotine withdrawal, mice, tryptophan, 5-HT, corticosterone, bupropion.

Submitted on 15-03-2024 – Revised on 08-10-2024 – Accepted on 09-10-2024

INTRODUCTION

Depression is a prolonged and varied illness demonstrating itself by mood and sleep disorder which may lead to suicidal thoughts. It is observed that depressed individuals have a higher tendency to smoke compared to non-depressed and the disruption of this dependence may result in more depression. Such a condition may be treated by the administration of anti-depressants and/or the use of nicotine replacement therapies. Bupropion Bup is an antidepressant and has been used to cure depression and affective disorder, it also supports smoking cessation. Nicotine addiction is the main obstacle to smoking termination. Smokers individually may not recognize the substantial risk of developing addiction, a realization often overlooked compared to their counterparts who smoke. Moreover, the symptoms of withdrawal in adults encompass heightened irritability, anxiety and feelings of depression. The neurotransmitter 5-hydroxytryptamine (5-HT) plays an important role in contributing to drug dependence (Muller and Homberg, 2015). A reduced serotonergic system is also linked to relapse after withdrawal (Kirby *et al*, 2011). An acceleration in the release of 5-HT can occur after nicotine administration. The assessment of stress response and nicotine addiction heavily relies on the significance of the hypothalamic-pituitary-adrenal (HPA) axis. Cholinergic receptors are notably present in the hypothalamus which are stimulated by nicotine, as a result, Corticotropic-releasing factors are released to stimulate cortisol production (Rohleder and Kirschbaum, 2006). Individuals who smoke cigarettes have been documented to experience persistent elevation in cortisol levels due to repeated exposure to nicotine, which is found in tobacco (Wong *et al.*, 2014). Certain individuals who smoke may use nicotine as a form of self-medication to alleviate feelings of depression (Clair. 2019). The antidepressant impact of nicotine is linked to the serotonergic system. Recent research indicates that nicotine has dual effects on the serotonergic system, displaying both anxiogenic and anxiolytic properties. Nicotine stimulates the release of 5-HT in several brain regions, (Seth *et al.*, 2002). Alterations in the serotonergic system, including serotonin-producing cells, transporters, and receptors, may affect smoking behavior (Tyndale, 2003). Additionally, a decline in serotonin levels occurs during nicotine withdrawal and a selective serotonin reuptake inhibitor counteracts the response to nicotine. (Ishikawa *et al.*, 1999).

Bup serves as a selective reuptake inhibitor for dopamine and noradrenaline, effectively alleviating cravings and other symptoms associated with nicotine withdrawal. Clinical trials have demonstrated that non-nicotine pharmacotherapy effectively aids smoking cessation. Specifically, Bupropion (Bup) is more likely to reduce depressive symptoms in highly nicotine-dependent smokers during active treatment**.** (Lerman *et al.,* 2004). Bup reduces noradrenergic neurons firing speed in the

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locus coeruleus, intensifying noradrenergic activity, and contributes to the alleviation of nicotine withdrawal symptoms. Additionally, Bup induces an abrupt functional blockade of human nicotine receptors. Bup also assembles an acute functional shutoff of human nicotine receptors (Krist *et al.,* 2021). Nicotine, the primary addictive element in tobacco smoke, primarily exerts its influence on the brain through action at the 5HT receptors, which are considered the most crucial site of its activity. Here, we present results from behavioral support in nicotine-withdrawn mice, a secondary role for diverse tryptophan (TRP) metabolism upon nicotine consumption plays a significant role in reinforcing dependence processes. Notably, individuals with comorbid psychiatric conditions exhibit substantially higher rates of tobacco dependence compared to the overall population, potentially indicating changes in tryptophan metabolism in psychiatric illnesses. Considering the involvement of 5-HT in the reinforcing properties of nicotine addiction, the current study was formulated to explore the impact of antidepressants on signs of nicotine abstinence and serotonergic neurotransmission in mice. Comprehending the involvement of tryptophan metabolism in psychiatric syndromes linked to increased rates of tobacco addiction may unveil fresh perspectives on underlying mechanisms of action of antidepressant Bup in nicotine reward and withdrawn mice on tryptophan metabolism.

MATERIALS AND METHODS

Chemicals and drugs

Bupropion hydrochloride was generously provided by the local Pharmaceutical Company and Nicotine hydrogen (+)-tartrate from Sigma while all other chemicals utilized were of the highest laboratory grade.

Animals and treatments

Animal procedures and treatments adhered strictly to the National Research Council guidelines for the care and use of laboratory animals (1996). Ethics approval was granted by the Board of Advanced Studies and Research (BASR /N0 0318/Sc.) University of Karachi, Pakistan. Adult male albino mice, weighing between 20-25g (n=6) were accommodated in each cage, subjected to a controlled light and dark cycle at 22±25°C and maintained with food and water (free access).

Drug preparation and administration

Bup hydrochloride was dissolved in 0.9% saline and the pH was adjusted to 7. Animals (n=24) were categorized into control and nicotine-withdrawn (NW) groups. Each group was further divided into saline and Bup treated (six mice/group). NW groups received nicotine-containing drinking water at a concentration of 3.08mg (equivalent to 1 milligram free base) per 100 ml of drinking water for four weeks, however, a control group of mice received nicotine-free water. To induce nicotine withdrawal, the water containing nicotine was replaced with regular drinking water. Bup (20mg/kg/ml) was injected 3.5 hours before the completion of the 72-hour withdrawal period control group received saline. Following the evaluation of withdrawal signs, all groups were euthanized through decapitation. Following decapitation, blood samples were collected in heparinized centrifuge tubes and clotted blood samples were then subjected to centrifugation (3000rpm) for 15 minutes to acquire plasma samples and stored at -20ºC until analysis. Whole brain samples were dissected within 30 seconds rinsed with saline and stored at -70º C till analysis.

Assessment of nicotine withdrawal somatic signs

The behavioral observations or nicotine withdrawal manifestations (such as shaking, scratching, chewing, and facial tremors) were conducted 30 minutes before and 3.5 hours after administering either Bupropion or saline. An observer blind to drug groups scored the somatic signs. Withdrawal was defined as a significant increase in total withdrawal symptoms as compared to the saline group at the same time point. A nicotine abstinence scale was used to assess behavioral signs and monitor the scoring of shaking, scratching, chewing and facial tremors every 5 minutes during the 30-minute examination enumerated by that scale. This scale, adapted from initial studies of nicotine withdrawal and borrowed from opiate and nicotine abstinence research, was used for the estimation of behavioral manifestations (Malin *et al*., 1992). After completing the behavioral evaluation, they were returned to their home cages.

Biochemical analysis

Plasma tryptophan

Plasma TRP levels were determined by the fluorimetric method of Denckla and Dewey (1967) was revised by Bloxam and Warren (1974). The principle of the test is based on the conversion of tryptophan to fluorophore, nor Harman, by heating under acidic conditions with formaldehyde and ferric chloride. The fluorescence, so developed, was read by using a spectrofluorimeter with excitation at λ373 and emission at λ452 nm respectively.

Plasma corticosterone

The steroid hormone corticosterone (animals) quickly develops a rather specific fluorescence in ethanolic sulphuric acid (1:3, v/v). The transformation of corticosterone into androst-4-ene-11ß, 17 dihydroxy-3-oxo17 carboxylic acid by the action of sodium hydroxide makes it much more fluorescent than corticosterone in sulfuric acid mixtures. Iso-octane is used as a washing agent for the steroid. The fluorescence was read by using a spectrofluorimeter with excitation at λ462 and emission at λ518 nm respectively (Glick *et al*., 1964).

Table 1: Effects of bupropion on brain indoles in nicotine-withdrawn mice

Parameters	Control		Nicotine Withdrawal		Two-way ANOVA df (1,20)		
	Saline	Bup	Saline	Bup	NW	Drug	NW× Drug
Brain TRP (µg/g)	2.35±0.13	2.85±0.08**	1.76±0.05††	2.82±0.1**	F=10.82 P<0.01	F=67.77 P<0.01	F= 8.63 P<0.01
Brain 5HT (ng/g)	0.66±0.07	0.71±0.05*	0.36±0.02††	0.81±0.08**	F=2.967 NS	F=19.12 P<0.01	F=12.38 P<0.01
Brain 5HIAA (ng/g)	0.45±0.04	0.31±0.01**	0.17±0.01††	0.26±0.01**††	F=57.88 P<0.01	F=1.59 NS	F=28.24 P<0.01
(5HIAA/5HT)	0.69±0.03	0.45±0.04**	0.48±0.04††	0.33±0.02*	F=22.19 P<0.01	F=30.50 P<0.01	F=1.63 NS

The mean values, along with their standard errors (SEM), were reported for each group of 6 rats. To assess statistical significance, we conducted a two-way ANOVA followed by the Newman-Keuls q-test. Notably, differences were marked as follows: *p<0.05 and **p<0.01 when comparing drug-treated groups to their corresponding saline groups, and †p<0.05, ††p<0.01 when comparing nicotine-withdrawn groups to similarly treated control groups.

Table 2: Effect of bupropion on plasma tryptophan and corticosterone levels

Assessments	Control		Nicotine Withdrawal		Two-way ANOVA df (1,20)		
	Saline	Bup	Saline	Bup	NW	Drug	NW× Drug
Plasma TRP (µg/ml)	15.72±0.65	17.82±0.43*	9.7±0.36††	9.8±0.55††	F=189.4 P<0.01	F =4.66 P<0.05	F=3.84 NS
Plasma Corticosterone (µg/dl)	46.72±1.1	59.0±2.06**	66±1.36††	57±2.36**	F=23.28 P<0.01	F=0.86 NS	F=35.05 P<0.01

The mean values, along with their standard errors (SEM), were reported for each group of 6 rats. To assess statistical significance, we conducted a two-way ANOVA followed by the Newman-Keuls q-test. Notably, differences were marked as follows: *p<0.05 and **p<0.01 when comparing drug-treated groups to their corresponding saline groups, and †p<0.05, ††p<0.01 when comparing nicotine-withdrawn groups to similarly treated control groups.

Table 3: Correlation matrix of plasma tryptophan and corticosterone with brain indoles in Bup treated nicotine withdrawn rats

Parameters	Brain TRP		Brain 5HT		Brain 5HIAA		Brain 5HT-Turnover	
	Coefficient (r)	p-value	Coefficient (r)	p-value	Coefficient (r)	p-value	Coefficient (r)	p-value
Plasma Tryptophan	-0.55	P=0.258	-0.846	P=0.033 P<0.05	0.385	P=0.451	0.385	P=0.451
Plasma Corticosterone	-0.823	P=0.044 P<0.05	-0.54	P=0.268	0.798	P=0.056	0.816	P=0.047 P<0.05

Table 3 depicts the significant negative correlations between plasma corticosterone versus brain tryptophan (r=-0.823, p=0.044) with a positive correlation versus brain 5HT Turnover (r=-0.816, p=0.047). Moreover, a significant negative correlation is observed between plasma tryptophan and brain 5HT (r=-0.846, p=0.033).

Neurochemical analysis

Homogenization of mouse brain (1-2g) was carried out in a tube containing 4.0ml of 0.1 M $HClO_4$ (400µl of l M $NaHSO_4$ added per liter). The brain was sonicated at 0-4ºC at a medium setting for 30-second periods using a power Sonic 603 Sonicator. 0.5 ml of 4.0 M $HClO_4$ was added and vortex mixing, the samples were then spun at 10,000g for 10 minutes and the clear supernatant was stored in polyethylene tubes for HPLC analysis.

High-performance liquid chromatography with a fluorescence detector was used to measure brain indoles (tryptophan, 5-HT and 5-hydroxyindole acetic acid (5-HIAA). The mobile phase was 0.01M sodium acetate, pH 4.5 and 15% methanol was passed through the stationary phase, using an octadecylsilane C18 separation column at a constant flow rate of 1ml/min, the detection was made with a fixed at 254nm excitation and 360nm emission wavelengths (Anderson *et al*., 1981).

STATISTICAL ANALYSIS

All data is presented as Mean ± SEM for all groups. Statistical analysis was performed using GraphPad Prism software (version 2.01), employing two-way ANOVA followed by post hoc Newman-Keuls q-test was used for group comparison and two-tailed t-test where appropriate. Significance was attributed to p-values less than 0.05. Pearson's correlation coefficient was also evaluated.

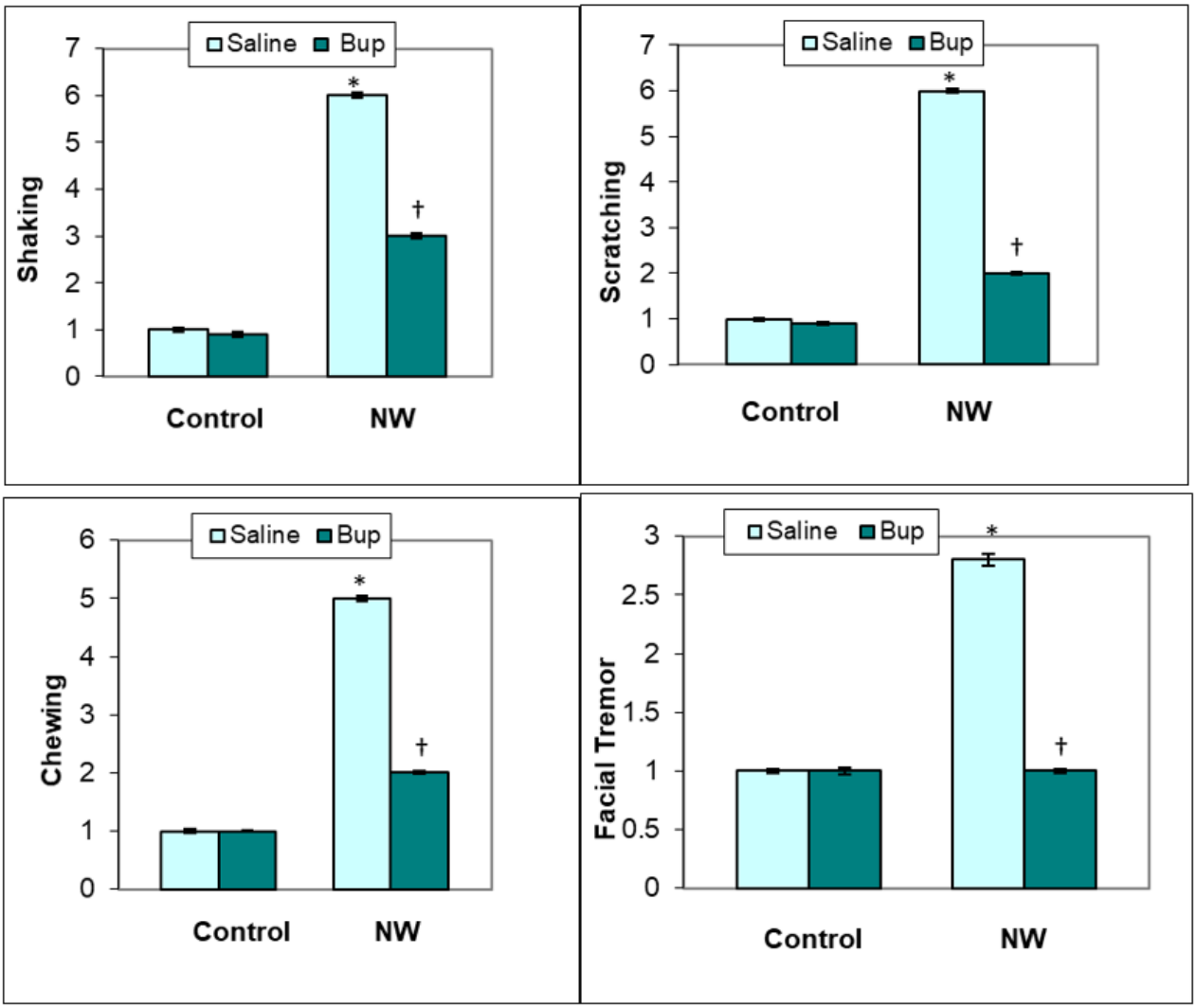

Fig. 1: Effects of Bupropion (Bup) on nicotine abstinence somatic signs in mice. The experiment was performed after 72 hours of nicotine withdrawal (NW). Somatic signs (occurrence) were monitored within each 5-minute interval for 30 minutes. Values are mean ± SEM of 6 mice per group. The saline control group was compared with the drug-administered and NW group. The Bup-administered NW group was compared with the Bup-administered group. Statistical analysis was performed using a student's t-test. The significance of the difference is indicated as follows: *p<0.001 and †p<0.001.

RESULTS

Table 1 Investigating the impact of Bupropion on brain indoles, we conducted data analysis using SPSS and employed a two-way ANOVA. Notably, Bup administration significantly increased brain tryptophan levels in both nicotine-withdrawn (NW) and control mice. Specifically, NW had a noteworthy effect ($F=10.82$, $P<0.01$). The drug itself showed a substantial impact ($F=67.77$, $P<0.01$). The NW x drug interaction was also significant ($F=8.63$, $P<0.01$). Brain serotonin (5HT) levels were elevated in both NW and control mice, with significant drug effects ($F=19.12$, $P<0.01$) and NW x drug interaction ($F=12.38$, $P<0.01$). Nicotine withdrawal had a significant impact on brain 5-hydroxyindoleacetic acid (5HIAA) levels. The statistical analysis showed a strong effect ($F=57.88$, $P<0.01$). The combination of nicotine withdrawal and drug administration also played a role. The interaction between NW and the drug had a significant effect on 5HIAA levels ($F=28.24$, $P<0.01$). Moreover, a profound reduction in brain 5HT-turnover was observed upon Bup administration in both NW and control mice with a significant effect of NW ($F=22.19$, $P<0.01$) and drug ($F=30.50$, $P<0.01$).

Table 2 demonstrates the effect of Bup on plasma tryptophan & corticosterone levels in NW mice. Data was analyzed using SPSS and the significance of differences was observed via two-way ANOVA. A profound reduction in plasma tryptophan levels was observed upon NW ($F=189.4$, $P<0.01$) which was found to be increased upon Bup administration in control mice only with the

effect of the drug (F=4.66, P<0.05). Moreover, a significant effect of NW (F=23.28, P<0.01) and NW x drug interaction (F=35.05, P<0.01) was observed on plasma corticosterone levels of control and NW mice.

Table 2 shows that NW had a profound impact on plasma tryptophan levels. The statistical analysis revealed a significant reduction (F=189.4, P<0.01) in tryptophan levels during NW. When Bup was administered, it had contrasting effects; in control mice (those not experiencing nicotine withdrawal), Bup increased plasma tryptophan levels. The drug effect was statistically significant (F=4.66, P<0.05). Both nicotine withdrawal and the drug interaction played a role in plasma corticosterone levels. NW had a significant effect (F=23.28, P<0.01). The interaction between NW and the drug also had a considerable impact (F=35.05, P<0.01).

Fig. 1 illustrates that Bup significantly reduced nicotine withdrawal signs in mice, evidenced by a decrease in the occurrence of chewing and scratching behaviors compared to the NW + Saline group. The experimental approach, outlined in the materials and methods section, employed a t-test for statistical analysis, table 3 depicts the significant negative correlations between plasma corticosterone versus brain tryptophan (r=-0.823, p=0.044) with a positive correlation versus brain 5HT Turnover ((r=-0.816, p=0.047). Moreover, a significant negative correlation is observed between plasma tryptophan and brain 5HT (r=-0.846, p=0.033).

DISCUSSION

The main effect of Bup is inhibition of dopamine and norepinephrine reuptake. In contrast, sustained bupropion administration (2 days) increases the firing activity of 5-HT neurons above normal, partly because it desensitizes the 5-HT_{1A} autoreceptor through an indirect noradrenergic mechanism. In addition, prolonged administration (14 days) of bupropion desensitizes the α_2-adrenergic heteroreceptors on 5-HT terminals (Ghanbari *et al*., 2011), thereby delineating their primary site of action on the NE system. It increases 5-HT neuronal activity, due to early desensitization of the 5-HT1A auto receptor (Costa *et al*., 2019) activity upon smoking cessation and reduction in these withdrawal symptoms upon Bup administration in rodents.

Previously, Bupropion (Bup) was identified as an antagonist at homopentameric 5-HT type 3A receptors. This study offers new insights into Bup's effects on 5-HT synthesis and turnover. Bupropion may cause high serotonin levels only when used with selective serotonin reuptake inhibitors (Sazakaly, 2008). However, there has been a reported case of serotonin syndrome caused solely by bupropion ingestion (Thrope *et al*., 2010). Earlier we have reported that an elevation of brain tryptophan contributes to the mechanism of action of serotonergic antidepressants (Bano *et al*., 2010). It was found that Bup alone increases the availability of tryptophan, 5HT though non-significant but decreases 5HT turnover (table 1) significantly these effects may contribute to its antidepressant and anxiolytic properties.

The present result shows nicotine withdrawal syndrome was reflected in shaking, scratching, chewing, and facial tremors in mice which were significantly attenuated in Bup-administered mice. (Fig. 1). Dysregulation of 5HT neurotransmission may also be one of the leading causes of this altered behavior as brain monoaminergic systems have been reported to be the pharmacological target to attenuate animal behavior (Napora *et al*., 2023). Nicotine consumption and withdrawal impact the dopamine mesolimbic system, which shares similarities with the effects of psychostimulant drugs. Studies reveal that repeated nicotine use leads to heightened sensitivity in 5-HT (2) receptor systems and reduced 5-HT turnover, correlating with the emergence of nicotine withdrawal symptoms (Yasuda, 2002)

The present result also shows a significant reduction in brain tryptophan 5HT levels upon NW (table 1). Research indicates that 5-HT3 receptors within the mesolimbic dopamine system play a role in the drug reward pathway. Additionally, 5-HT3 antagonists are proposed to modulate the effects induced by nicotine. (Zulkifli *et al*., 2019) However, 5-HT2A receptor antagonists and 5-HT2C receptor agonists have been implicated in effects similar to antidepressant drugs in NW rats, suggesting the involvement of 5-HT2 receptors**.** (Zaniewska *et al*., 2010).

A pronounced enhancement in plasma corticosterone levels and depletion in plasma tryptophan levels upon NW is seen probably due to the acceleration of tryptophan 2,3-dioxygenase (TDO) enzyme activity. It is suggested that nicotine withdrawal does not elicit any increase in plasma corticosterone concentration (Semba *et al.,* 2004). A previous study reported that the hyperactive hypothalamic-pituitary-adrenal axis can be corrected by drugs with antidepressant properties (Gupta *et al.,* 2014). This effect is also reflected in our findings upon Bup administration by a significant reduction in plasma corticosterone levels in NW mice. However, Bup tends to increase the depleted levels of plasma tryptophan in control mice only (table 2). Being a precursor, tryptophan in plasma also contributes markedly to the central 5-HT synthesis and an imbalance in the synthesis of 5HT leads to various neuropsychiatric disorders (Davidson *et al*., 2022). Tryptophan uptake by the brain rate under various conditions, not only depends on the concentration of tryptophan, but it also relies on the ratio of tryptophan to total neutral amino acids (Comai *et al.,* 2020). Dysregulation in the serotonergic system has also been

demonstrated to play a role in the onset of relapse after withdrawal (Kirby *et al*, 2011). Present results show that NW induces a significant reduction in brain tryptophan, 5HT, and 5HIAA levels which is again an endorsement of the elevated TDO activity upon NW. The activation of the HPA axis is the underlying factor in different stages of nicotine addiction (Heinrichs and Koob, 2004). In the present study, the lower brain 5HT concentration may be resulting from a reduction in the availability of circulating tryptophan to the brain and increased liver TDO activity which is correlated with elevated circulating corticosterone levels (Badawy *et al.*, 1989) that is again an indication of attenuation in HPA Axis regulation which in such a way reported as a target for treating depression (Menke., 2024). Elevated cortisol secretion occurs in depression, contributing to increased TDO activity, subsequently reducing serotonin synthesis and intensifying the depth of depressive symptoms. Previous studies indicate that elevated basal cortisol levels are linked to smoking due to the stimulation of nicotinic receptors (Mendelson *et al.*, 2008). The affinity for tryptophan of the transport system in the BBB equaled the albumin's affinity to alter tryptophan levels. However, tryptophan availability in the brain is better assessed by examining its levels via total tryptophan. Since albumin is not transported through BBB (Höglund *et al.*, 2019). Agents blocking or delaying this process are often clinically valuable drugs.

Bup administration increases brain TRP and decreases 5HIAA/5HT turnover in NW mice, possibly due to inhibition of 5HT reuptake. This effect is further endorsed by a notable positive correlation exists between plasma corticosterone levels and brain 5HT turnover as suppression in corticosterone induction by Bup tends to reduce 5HT turnover as well (table 3). These neurological benefits are attributed to its GABAergic and serotonergic activity; thus, it is supposed to be an anti-stress agent. Present results show that Bup administration to nicotine withdrawal mice increases 5HT concentration significantly. These findings are endorsed by another study suggesting Bup monotherapy to be effective and well-tolerated in patients with major depressive disorder (Tafseer *et al*., 2021). Bup is suggested in a study of combination therapy, to be an effective anti-smoking drug (Clark *et al.*, 2023). It selectively hinders the reuptake of dopamine and norepinephrine which may account for the induction of secondary effects on serotonergic receptors. The serotonergic effects of Bup have been a subject of debate, as certain human studies suggest that it does not possess serotonergic activity. Conversely, research in animals has indicated that Bup may exhibit certain serotonergic activity (Dong and Blier 2001; Piacentini *et al.*, 2003; El Mansari *et al*., 2008) with an elevation in hippocampal serotonin and other neurotransmitters in rats following Bup administration. These studies endorsed our findings of improving serotonin neurotransmission in NW mice upon Bup administration.

CONCLUSION

Taken together Bup has potential therapeutic effects on nicotine withdrawal-associated somatic signs by its ability to attenuate 5-HT turnover and plasma corticosterone in dependent mice. In addition, plasma corticosterone levels were positively correlated with brain 5-HT turnover in Bup-administered NW mice.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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