# Improved Serotonergic Tone Contributes to the Mechanism of Action of St John's Wort in Nicotine Withdrawn Mice

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**Abstract:** Present study aims to investigate the acute effects of St John's Wort (SJW) on nicotine withdrawal syndrome and serotonergic hypo activity in mice. Adult male Albino mice weighing 20-25g were housed 6 per cage under light and dark conditions at 22±3°C and maintained on lab chow and water *ad libitum* under standard housing conditions. Nicotine was administered at the concentrations of 3.08mg (1mg of free base) in 100 ml of drinking water for 4 weeks. Nicotine withdrawal was achieved by substituting nicotine containing water with drinking water. Nicotine withdrawn (NW) mice were evaluated for locomotor activity and abstinence signs at 72 h. Whole brain tryptophan (TRP). 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured using high performance liquid chromatography connected to fluorescence detector. St John's Wort (SJW) (500mg/kg) was given intraperitonially 3 h prior to completion of 72 h of nicotine withdrawal period. Behavioral analysis in SJW administered NW mice showed attenuation in nicotine abstinence signs (shaking, scratching, chewing and facial tremors) and locomotor activity when compared with respective controls. NW mice showed decrease in plasma TRP, brain TRP and 5-HT concentrations while increase in 5-HT turnover and corticosterone concentrations as compared to respective controls. SJW administrated NW mice showed decrease in corticosterone levels and 5-HT turnover while plasma TRP, brain TRP and 5-HT synthesis were increased when compared with similarly treated saline injected group. Our findings warrant SJW's therapeutic efficacy to alleviate nicotine withdrawal associated depression by virtue of its ability to improve serotonergic activity by increasing brain TRP, 5-HT concentrations and decreased turnover.

**Keywords:** Nicotine withdrawal, Mice, Tryptophan, 5-HT, St John's Wort.

## INTRODUCTION

St John's Wort (SJW), known botanically as *Hypericum perforatum* is a common medicinal plant. Emerging experimental and clinical studies indicate that SJW may be useful for the treatment of disorders originating from the central nervous system such as anxiety and depression. Many beneficial effects of SJW have been reported for stress related illnesses in clinical studies [1]. Recent research showed the effectiveness of SJW in treating other ailments, including cancer, inflammation-related disorders, eczema, burns, bacterial, viral diseases and as an antioxidant and neuroprotective agent [2].

Nicotine withdrawal syndrome can be produced in laboratory animals by discontinuing of nicotine after repeated administration of high doses leading to appearance of abstinence signs. The somatic manifestations of nicotine withdrawal in rodents can be detected as an increase in several stereotypic behaviors. Somatic signs of withdrawal include chewing, teeth-chattering, shakes, tremors, writhing, palpebral ptosis, gasps, and yawns [3].

Various serotonergic mechanisms may play complex or even opposing roles in nicotine withdrawal syndrome. Acute administration of nicotine increase serotonin release in brain [4] while spontaneous nicotine withdrawal reduces serotonin turnover in whole brain [5] conversely the serotonin precursor 5-hydroxy-tryptophan reversed withdrawal induced immobility in the forced swim test a putative model of depression in rodents [6], also stimulation of $5HT_3$ receptor may attenuate various serotonin features of nicotine withdrawal syndrome such as anxiety as indicated by conditioned placed aversion test [7]. This may suggest that serotonergic hypo activity may contribute to abstinence syndrome and renewed serotonin activity may alleviate this condition. In addition decrease serotonergic neurotransmission may elevate brain reward threshold and increase anxiety during nicotine withdrawal [8]. Enzyme monoamine oxidase (MAO-B) is decreased by smoking, which is responsible for the catabolism of several brain neurotransmitters, including dopamine, serotonin and noradrenaline [9]. Smokers have lower brain monoamine oxidase A and B activity, which normalizes during prolong abstinence as demonstrated by preclinical and clinical, studies [10]. It has been claimed that there is an association between cigarette smoking and the presence of suicidal behavior in smokers with depression due to lower brain serotonergic functions [11].

Some studies also indicated that SJW, an antidepressant agent, have some beneficial effects on ethanol withdrawal syndrome [12]. Indian Hypericum

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Perforatum has been reported as adaptogenic herbal medicine due to its anti-stress activity [13, 14]. Previous studies from our laboratory [15, 16] have elucidated the mechanism of action of SJW in forced swim test and have found its antidepressant mode of action. Keeping in view antidepressant and adaptogenic property of SJW it is desirable to investigate the acute effects of SJW on nicotine withdrawal induced alteration in serotonergic activity and circulating corticosterone levels in mice.

## ANIMALS AND TREATMENT

All animal procedures described below were conducted in strict accordance with the national research council for the care and use of laboratory animals (1996). Ethical approval was obtained from institutional animal ethics committee, University of Karachi. All efforts were made to minimize the number of animals and any pain/distress they might incur. Adult male albino mice weighing (20-25g) were housed in 6 per cages under light and dark conditions at 22±3$^{o}$C and maintained at lab chow and water *ad libitum* under standard housing conditions.

Mice were divided into two group saline and drug (SJW). Each group was further divided into two, control and nicotine withdrawn (NW) and each had six mice. Mice were given chronic nicotine orally in drinking water at the concentration of 3.08mg (1mg of the free base) in 100 ml of drinking water for 4 weeks. Control mice were given tap water. Nicotine withdrawal was achieved by replacing the nicotine containing water with drinking water. SJW was dissolved in a mixture of ethanol: saline, 1:3 v/v and was administered orally at the concentration of 500 mg/kg to mice 3h preceding to the completion of 72 h withdrawal period. Animals for behavioral study were different from those used for assessment of abstinence signs. All groups were killed by decapitation. Serum and brain samples were isolated and stored at -70$^{o}$ C until analysis.

### Locomotor Activity

The locomotor activity of the mice was monitored in an open field; sized 76 cm x 76 cm. The field had 16 equal divisions. Noise and light was maintained at a level not to disturb the mice causing changes in their locomotion, or inducing anxiety. The apparatus was cleaned with 70% alcohol after every mouse activity. The ambulation of mice is the counts of the number of inter line crossings during the 30 minutes session in the open field, with 2 minutes in the beginning for the proper settling of the mice in the apparatus before the actual counting [17].

### Nicotine Withdrawal Syndrome

#### *Abstinence Signs*

Nicotine withdrawal abstinence signs were recorded 30 minutes before and 3hrs after the administrations of antidepressant or saline. Antidepressant and saline treated mice were placed in clear cages to training for 30 min prior to each examination assembly and upon completion of the behavioral assessment they were returned to their home cage. For the estimation of the behavioral signs, a nicotine abstinence scale was compiled and utilized. The velocity of shaking, scratching, chewing and facial tremors during 30 min of examination was scored by abstinence scale. This scale was developed in initial studies and was adopted from opiate and nicotine abstinence studies [3].

#### *Plasma Analysis*

Trunk blood from decapitated mice was collected in heparinised centrifuge tube and plasma was isolated after centrifugation at 3000 g for 15 minutes. Plasma TRP [18] and corticosterone [19] levels were determined by the standard laboratory procedures.

### Neurochemical Analysis

The whole brain tissues were weighed and homogenized in 0.1M perchloric acid (1g in 4ml of 0.1M perchloric acid). The brains were sonicated at 0-4$^{0}$C at a medium setting for two 15-sec periods using a sonicator. After adding 0.5ml of 4M perchloric acid and mixing, the samples were spun at 10,000g for 10 min at 4$^{o}$C and a portion of supernatant was taken and stored at -70$^{o}$C until analysis. The analytical measurements for brain TRP, 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) were performed using high performance liquid chromatography connected to fluorescence detector. Mobile phase containing 0.01 M sodium acetate (pH 4.5 was adjusted with glacial acetic acid), 15% methanol was passed through the octadecylsilane separation column (25 cm in length 4.6 mm in diameter) at a constant flow rate (2 ml / min) with an operating pressure of 2000 – 3000 psi, using a 200 series pump. Fluorescence detection was performed on Shimadzu VT 03 detector at an operating potential of 0.8V.The flourimetric detector was used with a 254 nm excitation and 360 nm emission [20].

### Chemicals and Drugs

Nicotine Hydrogen (+)-tartrate was purchased from Sigma chemical Co and the SJW (standardized on

0.3% Hypericin contents) was a gift from Medics Laboratories, Karachi, Pakistan. All other chemicals were of highest analytical grade.

### Statistical Analysis

Data are expressed as mean± standard error of mean. Analysis was performed by using student's t-test. Difference between the two groups were considered significant when P<0.05.

## RESULTS

Figure **1** indicates that administration of SJW significantly decreases nicotine abstinence signs (shaking, scratching. chewing and facial tremors).

Figure **2** shows that nicotine withdrawal significantly increases locomotor activity by 41.7% (P<0.001) when compared with respective saline controls. The SJW administered mice demonstrated significant decreases by 30.8% (P<0.01) when compared with similarly treated saline controls. SJW pretreated nicotine withdrawal group of mice also showed 36.4 % (P<0.001) decrease when compared with similarly treated saline control in contrast increase in locomotor activity by 30.1% (P<0.01) was found when compared with respective controls.

Table **1** shows the effects of SJW on plasma TRP and brain indoles in nicotine withdrawn mice. Nicotine withdrawal group of mice showed significant decrease

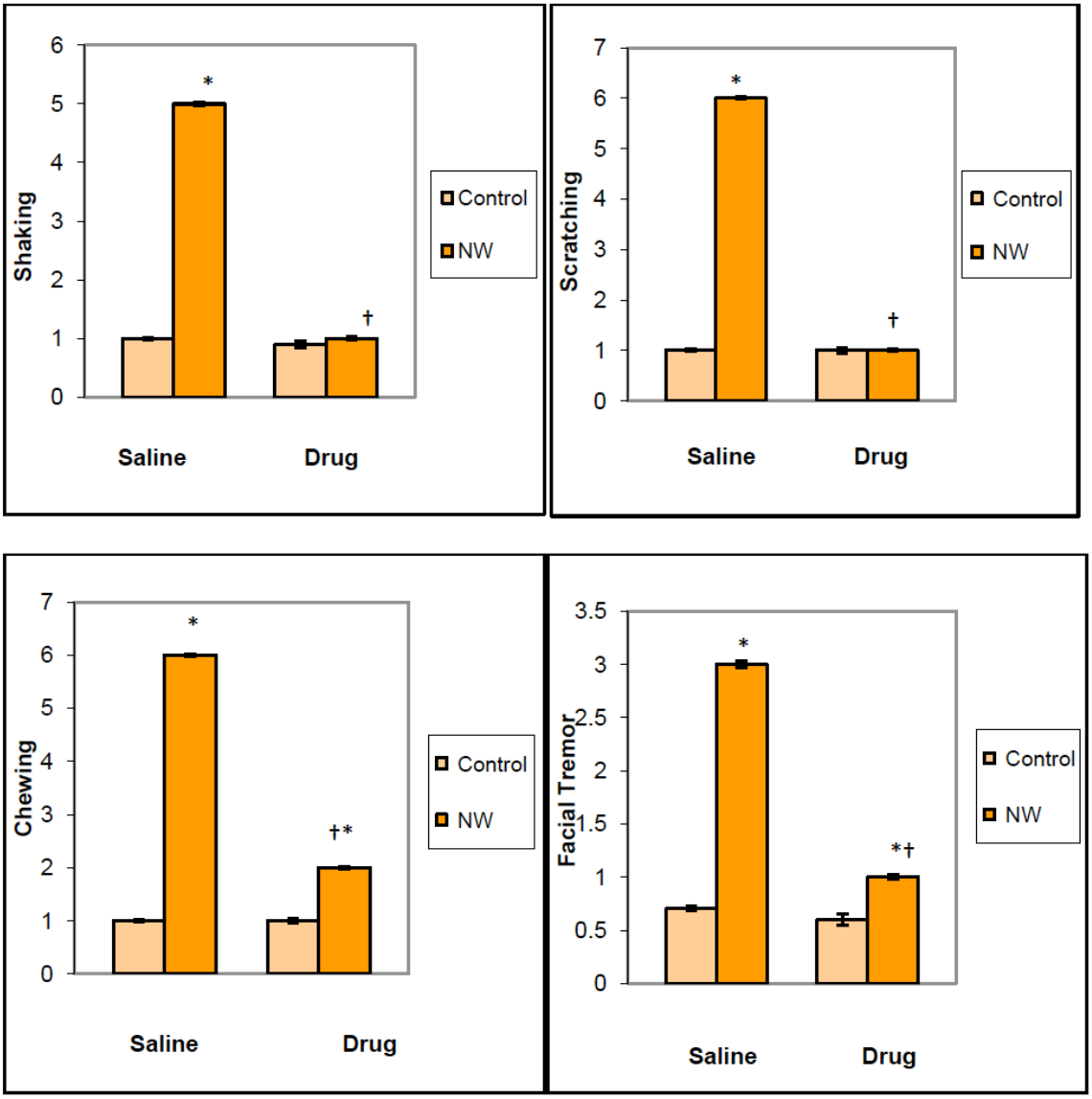


**Figure 1: Effects of SJW on nicotine abstinence signs in mice:** Experiment was performed after 72h of nicotine withdrawal (NW). All values are mean ± SEM of 6 mice per group. Statistical analysis was performed using student's t-test. The significance of difference is indicated by *P<0.001 when NW group is compared with respective controls and † P<0.001 when drug (SJW) treated group is compared with similarly treated saline injected mice.

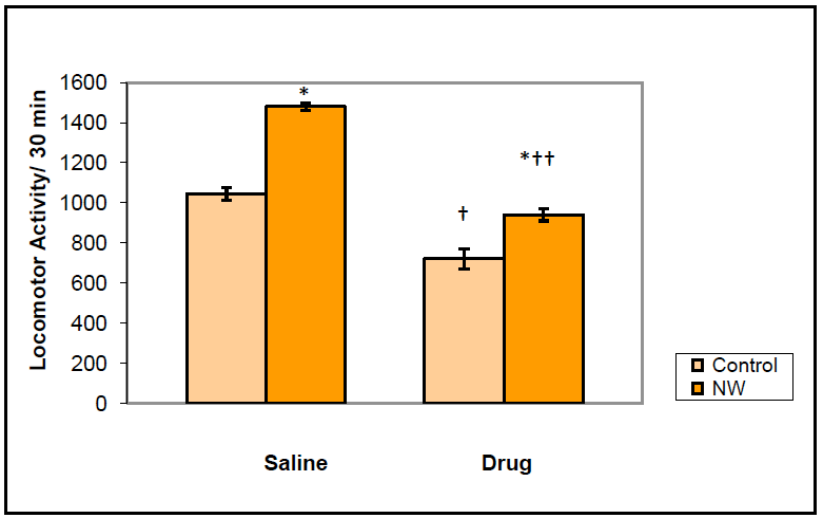


**Figure 2: Effects of SJW on behavior in open field test:** Experiment was performed after 72h of nicotine withdrawal (NW). All values are mean ± SEM of 6 mice per group. Statistical analysis was performed using student's t-test. The significance of difference is indicated by *P<0.001 when NW group is compared with respective controls and †P<0.01, †† P<0.001 when drug (SJW) treated group is compared with similarly treated saline injected mice.

in plasma TRP by 12.8% (P<0.05), brain TRP by 24.5% (P<0.01), 5HT by 26.3% (P<0.05), 5HIAA by 45.1% (P<0.01) and 5HIAA/5HT by 73.9% (P<0.01) when compared with respective controls. SJW treated mice demonstrated significant increase in total TRP by 23.7% (P<0.05), brain TRP by 72.5% (P<0.001) and 5HT by 14.4% (P<0.05) with no change in 5HIAA and 5HIAA/5HT turnover when compared with similarly treated saline controls. SJW pretreated nicotine withdrawn mice showed significant increase in plasma TRP, brain TRP and 5HT by 64.7% (P<0.05), 228% (P<0.001) and 82.1% (P<0.001) respectively while significant decrease in 5HIAA levels and 5HIAA/5HT ratio by 31.1% (P<0.001) and 62.5% (P<0.001) respectively when compared with similarly treated saline injected mice. SJW pretreated nicotine withdrawal group also showed increase in plasma TRP by 16% (P<0.01), brain TRP by 43.7% (P<0.001) and 5-HT by 17.2% (P<0.01) concentrations while significant decrease in 5HIAA by 11.4% (P<0.005) and 5-HIAA/5-HT ratio by 25% (P<0.001) when compared with respective controls.

Figure **3** shows that corticosterone concentrations were increased in nicotine withdrawn mice by 39.8% (P<0.001) when compared with respective saline controls while SJW pretreated nicotine withdrawal mice showed significant decrease in corticosterone concentrations by 22.5% (P<0.001) when compared with similarly treated saline injected group and by 22.5% (P<0.001) when compared with respective controls. SJW administered group showed significant increase by 14.5% (P<0.005) when compared with similarly treated saline injected group.

## DISCUSSION

Various experimental and clinical studies indicate that SJW can be useful for treating disorders originating from central nervous system. Antidepressants like action of SJW was reported in

**Table 1: Effects of SJW on Plasma Tryptophan and Brain Indoles in Nicotine Withdrawn Mice**

| Parameters | Saline | | Drug | |
|---|---|---|---|---|
| | Control | NW | Control | NW |
| Plasma TRP (ug/ml) | 10.1±0.4 | 8.8±0.36* | 12.5±0.39†† | 14.5±0.39†** |
| Brain TRP (ug/g) | 2.0±0.08 | 1.51±0.05* | 3.45±0.10†† | 4.96±0.12†††*** |
| Brain 5HT (ug/g) | 0.76±0.03 | 0.56±0.06** | 0.87±0.03† | 1.02 ±0.04††* |
| Brain 5HIAA (ug/g) | 0.31±0.02 | 0.45±0.01*** | 0.35±0.01 | 0.31±0.01†††* |
| Brain 5HIAA/5HT | 0.46±0.03 | 0.80±0.02*** | 0.40±0.01 | 0.30±0.01†††*** |

Experiments were performed 72h after withdrawal of chronic nicotine. All values are mean ± SEM of 6 mice per group. The significance of difference is indicated by *P<0.005, **P<0.01, ***P<0.001 when NW group is compared with respective controls and †P<0.005, ††P<0.01, ††† P<0.001 when drug (SJW) treated group is compared with similarly treated saline injected mice. Abbreviation used: TRP, tryptophan; 5HT, 5-hydroxytryptamine; 5HIAA, 5-hydroxyindoleacetic acid.

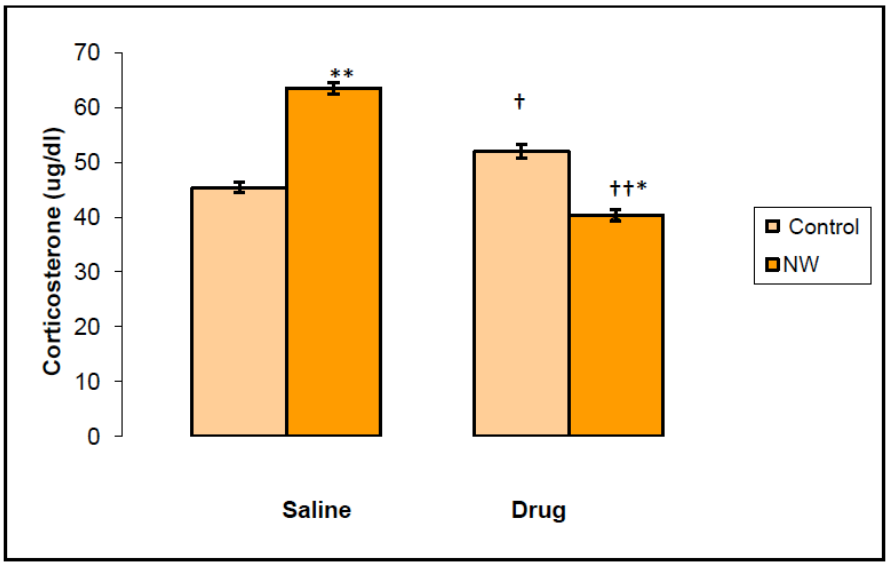


**Figure 3: Effects of SJW on corticosterone:** Experiment was performed after 72h of nicotine withdrawal (NW). All values are mean ± SEM of 6 mice per group. Statistical analysis was performed using student's t-test. The significance of difference is indicated by *P<0.01, **P<0.001 when NW group is compared with respective controls and †P<0.01, ††P<0.001 when drug (SJW) treated group is compared with similarly treated saline injected mice.

rodents [21] indicating its efficacy comparable to that of tricyclic [22]. The exact mechanism of antidepressant action is not clear but the effects have been related to monoamine oxidase inhibition / neurotransmitters reuptake.

Previously it has been shown that administration of SJW can attenuate nicotine withdrawal in mice [23]. It was shown that nicotine exposure produces changes in serotonergic system [4]. Studies have demonstrated the alleviative effect of SJW on stress-induced aggravation such as depression condition and cognitive impairment [21, 24, 25]. We have also found that administration of SJW can reduce the intensity of somatic symptoms of nicotine withdrawal (Figure **1**) that are in agreement with other studies [23, 26, 6]. Present study shows that SJW pretreated nicotine withdrawal mice showed decreased in hyper locomotion (Figure **2**). SJW causes significant depressant effect on locomotor activity at 500 mg/kg. Our results are consistent with those reported earlier [26]. Brain content of neurotransmitters such as noradrenaline, dopamine and serotonin were increased by SJW [27] and deficits in these neurochemicals have been considered to play a role in the expression of drug abstinence syndromes [28]. Thus it is possible to consider that SJW attenuate the nicotine withdrawal induced hyper locomotion in mice may be due to enhancing the functional tone of these neurotransmitters. Relationship between activity in serotonin neurons and motor function has been provided by studies experimentally inducing serotonergic hyperactivity and /or hypo activity. Reduced availability of 5-HT system in brain points to inhibitory role of 5-HT system in nicotine withdrawal induced hyper locomotion. This is further supported by studies of nicotine withdrawal that showed 5-hydroxytryptophan attenuates somatic signs of nicotine withdrawal [29].

Present results show that plasma TRP were significantly increased in SJW administered mice when compared with similarly treated saline controls and in SJW pretreated nicotine withdrawal group when compared with respective controls (Table **1**). Increased plasma TRP leads to corresponding increase in brain serotonin synthesis and thus turnover. Tryptophan is an essential amino acid that serves as the precursor for the biogenic amine serotonin [30]. In the liver, the amino acid tryptophan (TRP) is catabolized by the enzyme tryptophan-2, 3-dioxygenase (TDO). Previous studies from our laboratory have shown that acute SJW administration (10mg/kg) increases tryptophan availability to the brain secondarily to inhibition of TDO enzyme activity [31]. Inhibition of TDO enzyme gene expression following SJW treatment in rats was also shown by our laboratory [32].

Serotonin is synthesized from TRP in the brain. Tryptophan hydroxylase is the rate-limiting enzyme in 5-HT biosynthesis. One way to increase serotonin in the brain would be to increase the amount of TRP available for uptake to the brain. This could be made possible by inhibiting hepatic TDO activity [33].

However, the transportation of TRP to the brain is dependent on a carrier system to cross the blood-brain barrier. There is also competition between the neutral amino acids for attachment to this carrier molecule, thus affecting the amount of TRP getting into the brain [34].

Present study shows that plasma tryptophan concentration was significantly decreased in NW mice (Table **1**). This decrease in plasma tryptophan could be due to enhancement of TDO activity by a hormonal type mechanism. The increase in TDO activity characterized the hormonal induction mechanism and this is supported by the observed increase in plasma corticosterone concentrations in present study, similar findings have been reported during ethanol withdrawal in rats [35]. Plasma corticosterone concentration has also been shown to increase during withdrawal of opiates [36] and nicotine [37]. In contrast, a study showed nicotine withdrawal lowers corticosterone levels during restraint stress suggested sub sensitivity of the hypothalamic-pituitary-adrenal axis (HPA axis) to stress. This may be implicated in the precipitation of depression during smoking cessation [38]. By some estimates, 50%–60% of patients with major depression also suffer from nicotine dependence [39]. The prevalence of depression among smokers has been estimated at three times that of nonsmokers. Smoking may be used as self-treatment for depression; consequently, smoking cessation could lead to depression, which increases the risk of suicide [40].

The present data shows that corticosterone levels were increased in SJW treated and nicotine withdrawn mice. Our results are in agreement with previous finding that also showed increase corticosterone levels in SJW administered rats and nicotine withdrawn rats [16, 35]. However SJW pretreated nicotine withdrawn mice showed decreases in corticosterone levels when compared with respective drug control group. The reduction in corticosterone by SJW administered nicotine withdrawn mice is liable to the mechanism that restores negative feedback mechanism on several levels of the HPA axis resulting in reduced release of corticotropin-releasing hormone and adrenocortico-tropic hormone. Hence cortisol binds to receptors at the level of the hippocampus, hypothalamus and pituitary to mediate negative feedback to the HPA axis [41, 42].

Many studies have demonstrated that adrenalectomy and replacement of corticosterone or the administration of corticosterone exogenously influences tryptophan hydroxylase activity and 5HT turnover in the brain [43]. However, corticosteroids may also act to modulate serotonergic neurotransmission directly by regulating 5-HT receptors. Increased brain serotonin levels improve the ability to cope with stress, while a decline in serotonin activity is associated with depression. Changes in brain tryptophan level are parallel changes in the rates at which serotonin is synthesized and released from raphe nucleus neurons. The enzyme tryptophan hydroxylase which catalyzes tryptophan conversion to 5-hydroxy tryptophan (the intermediate in serotonin synthesis) is highly unsaturated with it substrate, hence any increase or decrease in intraneuronal tryptophan levels apparently will cause a parallel change in the enzyme's net activity. Present results show that brain TRP and 5HT concentrations were increased while 5HIAA and 5HIAA/5HT turnover were decreased by SJW administered mice (Table **1**). A decrease in 5-HIAA levels predicts increased 5-HT release as SJW act as MAO inhibitor has not been established [44].

We have found that during nicotine withdrawal brain TRP, 5HT and 5H1AA concentrations were decreased (Table **1**) may be due to decreased availability of tryptophan to the brain concentration secondarily enhancement of TDO by increased circulating corticosterone concentrations. There is also evidence that 5-HT neurotransmission is involved in the mediation of nicotine dependence. Chronic nicotine treatment decreases the concentration of 5-HT in the hippocampus and increases the number of hippocampal 5-$HT_{1A}$ receptors [37]. During nicotine abstinence, decreased 5-HT, combined with up regulated $5HT1_A$ receptors, may contribute to symptoms of depression [45]. Decreased 5HIAA concentration may be due to low baseline MAO activity significantly predicted the intensity of craving and anxiety reported after smoking cessation, suggesting that there was an association between severity of affective symptoms often cigarette withdrawal and extent of MAO inhibition [46, 47]. Anxious depression is characterized by the decrease in serotonergic activity [48] and possibly by the decrease in serotonin concentration in the synapses. Several studies have described the emergence of depressed mood during the nicotine withdrawal period; two of these studies, one based on a clinical sample [49], the other based on an epidemiological sample of young adults [50], found that post cessation depressed mood occurred more frequently among smokers with a history of major depression. The onset of emotional states resembling full-blown major depression during the nicotine withdrawal period has also been reported [51, 52].

## CONCLUSION

Our findings warrant SJW's therapeutic efficacy to alleviate nicotine withdrawal associated depression by virtue of its ability to improve serotonergic activity by increasing brain tryptophan and thus 5-HT concentrations and decreased 5-HT turnover (5-HT/5-HIAA ratio).

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