ST. JOHN'S WORT MODULATES BRAIN REGIONAL SEROTONIN METABOLISM IN SWIM STRESSED RATS

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ABSTRACT

Present study has investigated acute effects of Saint Johns Wort (SJW, 500mg/kg) administration on behavioral, neuroendocrine responses and serotonergic activity following forced swim test (FST) exposure in rats. The results show that SJW increased swimming and climbing behaviour of rats during FST exposure. Swim stress produced significant reduction in serum total tryptophan ($P<0.01$), increase in corticosterone ($P<0.01$) and 5-hydroxytryptamine (serotonin, 5-HT) turnover in hypothalamus by 100% ($P<0.01$), amygdala by 148 % ($P<0.01$), and hippocampus by 41% ($P<0.05$) when compared with unstressed saline injected group. SJW in swim stressed rats when compared with saline injected stressed rats altered neither lowered serum tryptophan nor enhanced HPA axis response, however 5HT was found to be increased by 110% ($P<0.01$), 163% ($P<0.01$) and 172% ($P<0.01$), in hypothalamus, amygdala and hippocampus respectively. 5-hydroxyindoleacetic acid (5HIAA) was also found to be increased in hypothalamus by 74% ($P<0.01$), amygdala by 45% ($P<0.01$) and hippocampus by 143.5% ($P<0.01$). Further SJW administration in unstressed rats showed decrease in tryptophan ($P<0.01$), increase in corticosterone ($P<0.01$), 5HT was found to be decreased in hypothalamus (47%, $P<0.01$) and in amygdala (13 %, $P<0.05$) with no change in hippocampus, while 5HIAA was found increased in hypothalamus by 58 %($P<0.01$), amygdale by 203 % ($P<0.01$) and hippocampus by 171% ($P<0.01$). The data shows that SJW affects circulating tryptophan and corticosterone in absence of conditioned stress but not in its presence. In conclusion, SJW increases intraneuronal 5HT metabolism but inhibits its release under adverse conditions proving its anxiolytic property. Thus, these effects produced by the SJW add to our understanding of the interactions between SJW and stress induced behavioral, neuroendocrine and serotonergic alterations.

Keywords: Forced swim stress, behaviour, St. John's Wort, 5-HT, corticosterone.

INTRODUCTION

Forced swimming test has been used widely for screening of substances with the potential antidepressant effects. It is a non-escapable stressful situation in which rats are forced to swim and become immobile after vigorous activity. The act of their immobility is quantified as a behavioral despair (Porsolt *et al.,* 1978), that shows symptoms similar to clinical depression with respect to etiology and responsiveness to antidepressant treatment and therefore it is considered as an animal model of depression (Borsini and Meli, 1988). Forced swimming test (FST) has shown to produce remarkable changes in extra cellular 5HT than other environmental stressors such as tail pinch, forced locomotion, immobilization and cold (Kirby and Lucki, 1997). Micro dialysis studies have shown that forced swim test produces regional specific changes in 5HT neurotransmission. As it was shown that FST increased levels of 5HT in the striatum while decreased 5HT levels in amygdala and lateral septum, however hippocampus and frontal cortex did not show any alteration in 5HT levels (Kirby *et al*., 1995). Elevated levels of corticosteroids (principally cortisol in humans and corticosterone in rats), has been reported most commonly in major depressive disorders and are known to be regulated by the neuroendocrine control of the HPA axis in the hypothalamus (Garcia *et al.*, 2000). A number of experiments have proved that hypothalamic 5-HT enhances HPA axis secretory activity in stress through 5HT1A and 5HT2 receptors and suggest that corticosteroids also accelerate 5-HT synthesis and turnover (Chalmers *et al.*, 1993). The brain regions that are involved in regulating mood, behavior, and emotions are important in monitoring impulsivity against aversive stimuli. Hippocampus and amygdala are the important limbic brain structures where antidepressant drugs have been shown to cause both acute and regulatory effects (Blier *et al*., 1990; Duncan *et al*., 1986). Hippocampus has been reported to be a site of serotonergic innervations that involves in regulating mood, behavior and learning abilities (Warner-Schmidt and Duman, 2006). The role of amygdala in depression is to decrease the sensitivity to activating stimuli in conditioning fear. 5-HT within the amygdala may also modulates pathways involve in aggression and impulsivity, suggesting its possible role in stress and depression (Ressler and Nemeroff, 2000). Further, cell death in brain regions, loss of memory and atrophy has been reported in untreated clinical depression (Duman, 2002). Cerebral 5HT synthesis is controlled mainly by the availability of tryptophan and its depletion

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in the serum is a more generalized marker in depression (Cowen, 2002). A number of antidepressants have been worked out that increase the availability of tryptophan to the brain (Badawy and Evans, 1981; Bano and Sherkheli, 2003). Efforts in an attempt to eliminate stressed induced complications (Connor *et al*., 1997; Dunn 1988; Inoue *et al*., 1994) in animal models, have classified antidepressants accordingly depending on their response and mechanism of action (Poleszak *et al.,* 2006; Connor *et al*., 1999). Long and short-term treatments of various tricycle antidepressants (TCA's), monoamine oxidase inhibitors (MAOI's), various selective serotonin reuptake inhibitors (SSRI's) and some newer antidepressants are under investigation to determine their mechanism of action and side effects in depressive therapy. Hypericum Perforatum (St. John's Wort) has recently been developed as an alternative medicine to treat depression and anxiety. Hypericum extract is a pharmacological agent obtained from hypericum perforatum (Miller, 1998). Its alcoholic extract hyperforin is important to inhibit the synaptic uptake of serotonin, NA and DA with almost equal potency (Muller *et al.,* 2001), whereas hypericin has little affinity for monoamine uptake, though its effects are also important for antidepressant action (Shulte- Lobert. *et al*., 2004). Hypericum contains numerous compounds with various biological activities *and* it is not known whether one or a combination produces the antidepressant effects of Saint John's Wort (SJW). Previous studies in our laboratory (Bano and Dawood 2008), on whole brain have elucidated the mechanism of action of SJW on serotonergic modulations in FST and suggested it's anti anxiety mode of action. Present study aims to investigate the acute effects of SJW on FST induced behavioral, serotonergic changes in various brain areas (hypothalamus, amygdala, and hippocampus) and peripheral changes in circulating tryptophan and corticosterone levels.

MATERIALS AND METHODS

Animals and treatment

Adult male (Albino Wister) rats (weighing 150-200 gm) were used throughout the study. All animals were housed 5 per cage under light and dark conditions at 25 $\pm$ 2^{o}C and maintained on lab chow and water *adlibidum* under standard housing condition. Rats were divided into two groups (Saline and Drug) containing 10 rats in each. Saline (0.95% NaCl) and drug (SJW) treated group of rats were further assigned for unstressed and stressed containing 5 in each group. SJW was dissolved in a mixture of dimethylformamide (DMF): saline, 1:3 v/v and was administered orally at a dose of 500mg/kg /ml /body weight. Animals that were exposed to swim stress procedure were decapitated 5 min after exposure on day 2 of the test whereas unstressed animals after 3.5 hours of drug / vehicle administration.

Forced Swim Test (FST) and Behavioural analysis

Animals were exposed to forced swim test as described in detail (Bano and Dawood, 2008). Behavior during test swimming session was scored using a time sampling method (Detke *et al.*, 1995). Every five seconds; one of three behaviors was recorded. Immobility was scored when the animal was making the maximum movements necessary to stay afloat. Swimming was scored when the animal actively swam around the tank, making movements greater than those necessary to stay afloat. Climbing was scored when the animal made vigorous thrashing movements with its forepaws, usually directed against the sides of the tank. Behavioral results are shown as the total number of counts for each behavioral category.

Serum analysis

Trunk blood was collected and centrifuged at 4000rpm for 30minutes.The serum was collected and frozen at –70 until analysis. Serum tryptophan and corticosterone levels were determined by the standard procedures (Bloxam and Warren, 1974; Glick *et al.,* 1964), respectively.

Neurochemical analysis

After decapitation, the brains were rapidly removed and hypothalamus, amygdale and hippocampus were isolated. After weighing the respective regions, the tissues were homogenized and deprotienised in 5 volumes of extraction buffer containing 0.4M perchloric acid, 0.1% sodium metabisulfide, 0.1% ethylene- diamine tetra acetic acid (EDTA) and 0.01% cysteine. After centrifugation the supernatants were stored at -70. The analytical measurements were performed by high performance liquid chromatography with electrochemical detector. A reverse phase chromatography was used in all analysis of 5-HT and its metabolite 5-hydroxyindolacetic acid 5HIAA.The ratio of 5HIAA/5HT was used as an index of 5HT turnover. The mobile phase consisted of methanol (18%), octyl sodium sulfate (0.03%), & EDTA (0.005%) in a 0.1M sodium phosphate buffer, pH 2.9 was passed through the ODS separation column (25 cm in length 4.6 mm in diameter) at a constant flow rate (1 ml / min) with an operating pressure of 2000 – 3000 *psi*, using a 200 series pump. Electrochemical detection was performed on Perkin Elmer VT 03 detector at an operating potential of 0.8V (Kenett *et al*., 1985).

Drug and chemicals

The drug Saint John's Wort was gift from local pharmaceutical (Medic's laboratories Pakistan) company. All other chemicals were of highest analytical grade.

STATISTICAL ANALYSIS

The data, presented as means $\pm$ SD (n=5) for each data point. P values less then 0.05 were considered significant.

RESULTS

Fig. 1 shows significant effects of SJW on behavior of rats in FST. It decreased floating time by 87.80% *(P<0.001)*, increased swimming by 112.2% *(P<0.001)* and increased climbing behavior of rats by 390.10% *(P<0.001)*.

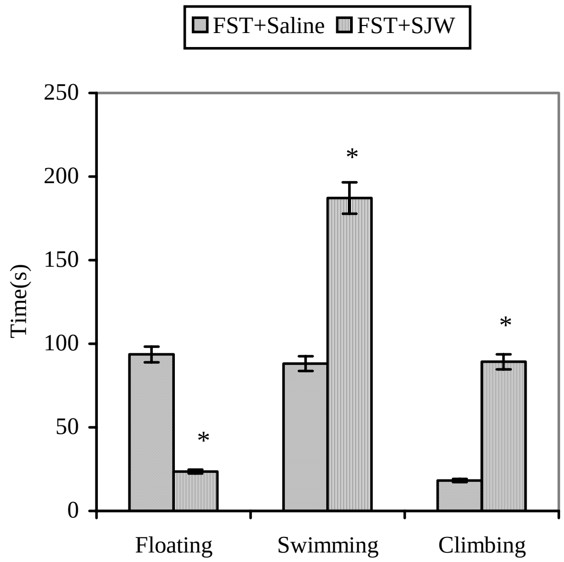

Fig. 1: Effects of administration of SJW 500mg/kg on floating, swimming, and climbing behavior of rats. Experimental details are given in material and methods. All values, presented as means ± SEM of five rats. Statistical analysis was performed using student's *t*-test. The significance of difference is indicated by *P<0.001.

Table (1) shows the effects of SJW on FST induced brain regional 5HT and 5HIAA metabolism. Statistical analysis by two way analysis of variance ANOVA in the table, shows significant effect of FST on 5HT levels in hypothalamus *F=23.70 (P<0.01)*, amygdala *F=10.54 (P<0.01)*, and in hippocampus *F=29.48 (P<0.01)*. Similarly FST did not show any significant effect on 5HIAA levels in hypothalamus, however amygdala and hippocampus both showed significant effect *F=223.19 (P<0.01), F=8.00 (P<0.05)* respectively. No significant effect of FST on 5HT turnover (5HIAA /5-HT ratio) was found in the hypothalamus, however, amygdale *F=8.44 (P<0.05)* and hippocampus *F=27.01 (P<0.01)* showed significant increase in 5HT turnover. SJW showed significant effects on 5HT levels in the hypothalamus *F=13.84 (P<0.01)*, the amygdala and hippocampus also showed significant effects *F=67.95 (P<0.01), F=220.23 (P<0.01)* respectively. SJW on 5HIAA levels showed significant effects on hypothalamus *F=95.17 (P<0.01)*, amygdala *F=744.3 (P<0.01)* and hippocampus *F=165.98 (P<0.01)*. The drug SJW also showed significant effects on 5HIAA/5-HT ratio on hypothalamus *F=6.9 (P<0.05)*, amygdala *F=34.15 (P<0.01)*, and in hippocampus *F=55.85 (P<0.01)*. FST x drug interaction showed significant effects on 5HT levels in hypothalamus, amygdala and hippocampus *F=756.69 (P<0.01)*, *F=140.48 (P<0.01)*, *F=238.01 (P<0.01)* respectively. 5HIAA levels by FST x Drug did not show any significant affect in the hypothalamus and hippocampus, while produced significant effect on amygdala significantly *F= 282.2 (P<0.01)*. FST X drug interaction on 5HT turnover on hypothalamus *F=35.86 (P<0.01)*, amygdala *F = 231.04 (P<0.01)*, and hippocampus *F = 78.71 (P<0.01)*, was found significant.

Post hoc analysis by Newman Kuels test showed significant effects of FST on 5-HT (*P<0.01)* in the hypothalamus, amygdala *(P<0.01)* and in hippocampus *(P<0.01)*. There was no significant effect of FST on 5HIAA in the three regions when compared with (saline injected unstressed group), however significant effect was found on 5HT turnover, that seemed to be increasing in all the three regions of the brain, with the significant values of *(P<0.05)* in hypothalamus, Amygdala *(P<0.01)* and in hippocampus *(P<0.05)*. Saint John's Wort injected swim stressed rats when compared with saline stressed rats showed marked increase in 5-HT levels in the hypothalamus *(P<0.01)*, amygdala *(P<0.01)*, and in hippocampus *(P<0.01)*. 5HIAA levels were also found to be increased significantly in the hypothalamus *(P<0.01)*, amygdala *(P<0.01)* and in hippocampus also *(P<0.01)*. In this comparison 5HIAA/5-HT turnover did not show any significant difference because of the increase in both 5-HT and 5HIAA levels. When control (saline) group was compared with SJW drug control, significant decrease in 5-HT was found in hypothalamus *(P<0.01*), amygdale (*P<0.05*) while no significant change was found in 5-HT in hippocampus. 5HIAA levels were found to increase significantly in the hypothalamus *(P<0.01*), amygdale *(P<0.01)* and in hippocampus *(P<0.01)*. Similarly 5HIAA/5-HT ratio was significantly higher in hypothalamus *(P<0.01)*, amygdale *(P<0.01)* and in hippocampus *(P< 0.01)*. Drug treatment increased 5HIAA levels in both cases (in drug control and in FST group with drug), showing Saint John's Wort is active in metabolism of 5-HT (increasing 5HT turnover).

Table 2 shows the effects of Saint Johns Wort (SJW) on serum tryptophan and corticosterone levels. Data analyzed by 2-way ANOVA in the table shows significant effect of stress (FST) on serum tryptophan and corticosterone levels *F=8.62 (P<0.01)* and *F=53.98 (P<0.01)* respectively. The effects of drug (SJW) on serum tryptophan and corticosterone concentrations *F= 10.14 (P<0.01)* and *F=115.62 (P<0.01)* were also significant. Interaction between FST X Drug was significant in serum tryptophan *F=19.4 (P<0.01)* and as well as on corticosterone levels *F=88.21 (P<0.01)*.

Table 1: Effects of SJW on forced swim test exposure on brain regional 5HT and 5HIAA concentrations

	Concentration in (ng/gm wet wt of tissue)	Saline		Drug		Two way ANOVA DF 1,16		
		Un Stressed	Stressed	Un Stressed	Stressed	FST	Drug	FST x Drug
Hypothalamus	5HT	1591.6 ± 39.9	885.8 ±33.15**	850± 18.81++	1859 ±25.91++**	23.70 P<0.01	13.84 P<0.01	756.69 P<0.01
	5HIAA	754.5 ±76.6	818.21 ±10.60**	1188.1 ±62.25++	1420 ±117.83++*	3.68 NS	95.17 P<0.01	1.19 NS
	5HIAA/5HT	0.46 ±0.04	0.92± 0.04*	1.39± 0.07++	0.77 ±0.05**	0.0059 NS	6.9 P<0.05	35.86 P<0.01
Amygdala	5HT	923.2 ±20.7	415± 26.57**	801.79 ±20.55+	1091 ±54.46++**	10.54 P<0.01	67.95 P<0.01	140.48 P<0.01
	5HIAA	485.6 ±21.87	527.8± 0.41	1471.7 ±14.08++	763.45 ±37.01++**	223.19 P<0.01	744.3 P<0.01	282.2 P<0.01
	5HIAA/5HT	0.52 ±0.02	1.29 ±0.09**	1.83 ±0.06++	0.70 ±0.04**	8.44 P<0.05	34.15 P<0.01	231.04 P<0.01
Hippocampus	5HT	823.58 ±11.30	527.66 ±12.55**	805.57 ±29.10	1433.6 ±49.91++**	29.48 P<0.01	220.23 P<0.01	238.01 P<0.01
	5HIAA	485.2 ±21.47	431.44 ±5.5	1314.46 ± 49.20++	1050.2 ±98.11++**	8.00 P<0.05	165.98 P<0.01	3.51 NS
	5HIAA/5HT	0.58 ±0.02	0.82 ±0.04*	1.63 ±0.10++	0.73 ±0.07**	27.01 P<0.01	55.85 P<0.01	78.71 P<0.01

Experimental details are given in material and methods section. Statistical analysis was performed using two ways ANOVA followed by Newman Keul's test. All values are means ± for each group of five rats. The significance of difference is indicated by **P<0.01, *P<0.05 when stressed group was compared with respective unstressed group. And, ++P<0.01, +P<0.05, when SJW injected group was compared with similarly treated saline treated group.

Table 2: Effects of SJW on serum tryptophan and corticosterone concentrations

Parameters	Saline		Drug		Two way ANOVA DF 1,16		
	Unstressed	Stressed	Unstressed	Stressed	FST	Drug	FST x Drug
Serum Tryptophan (ug/ml)	25.42 ±0.71	17.41** ±0.5	17.16++ ±1.24	18.75 ±1.4	8.62 P<0.01	10.14 P<0.01	19.48 P<0.01
Corticosterone (ug/dl)	44.03 ±1.37	82.45** ±1.23	90.27++ ±2.47	85.58 ±3.40	53.98 P<0.01	115.62 P<0.01	88.21 P<0.01

Experimental details are given in material and methods section. Statistical analysis was performed using two ways ANOVA followed by Newman Keul's test. All values are means ± for each group of five rats. The significance of difference is indicated by **P<0.01, *P<0.05 when unstressed group was compared with respective stressed group. And, ++P<0.01, +P<0.05, when SJW injected group was compared with similarly treated saline treated group.

Post hoc analysis by Newman keuls test shows significant decrease due to FST on serum tryptophan *(P<0.01)* and increase in corticosterone concentrations *(P<0.01).* Administration of SJW also shows significant decrease in serum tryptophan *(P<0.01)* and increase in corticosterone concentrations *(P<0.01),* when compared with saline injected rats. There was no significant effect of SJW on swim stressed rats when compared with similarly treated swim stressed rats on serum tryptophan as well as on corticosterone levels.

DISCUSSION

Present study shows that SJW administration increased both swimming and climbing behavior of rats in FST. These effects produced by pretreatment of SJW in FST may indicate that it antagonizes stress induced emotional behavior, that has also been confirmed by other investigations (Butterweck, 2003; Butterweck *et al*., 2003) It has been reported earlier that antidepressants that possess the ability to enhance serotonergic neuro-transmission increase swimming behavior of rats, while those having effect in inducing catecholaminergic response also increase climbing behavior of rats (Cryan *at al*., 2005). SJW, increased both swimming and climbing behaviour of rats in the present study and may possess the ability to enhance both serotonergic and catecholaminergic neurotransmission. Detke and coworkers have found the ability of SJW effective in climbing behavior of rats. These authors suggest that SJW may share its antidepressant property with norepinephrine reuptake blocker (Detke *et al*., 1995). Therefore it is not unreasonable to suggest that SJW antagonizes depressive like behaviour and may possess effective therapeutic property.

The data presented here, shows that administration of SJW did not produce any significant change in stress-induced increase in 5-HT turnover in hypothalamus, amygdala and in hippocampus, however increased both the levels of 5HT and 5HIAA in the all the three regions. In parallel to our findings, administration of hypericum extract containing 0.3% hypericin and 4.5% hyperforin in FST has shown to enhance serotonin, nerepinephrine and dopamine contents in the cortex, diencephalon, and brain stem (Calapai *et al*., 2001). In contrast to our present findings, previous studies on antidepressants as tricyclic antidepressants (TCAs) desipramine, selective serotonin reuptake inhibitors (SSRIs) paroxetine, and serotonin nor-adrenaline reuptake inhibitors (SNRI's) venlafexine, have been shown to attenuate FST induced increase in serotonergic turnover (Connor *et al*., 2000; Connor *et al*., 1999). These studies reported that antidepressant-induced attenuation of FST-related increase in serotonin turnover is not an indicative of behavioral activity in this test. The inability of SJW to attenuate swim stress induced increase in 5-HT turnover in the present findings may represent its mechanism of action other than classical antidepressants that is also inconsistent with its behavioral activity in FST paradigm.

An acute administration of SJW in the present findings in unstressed rats has caused an increase in 5-HIAA in all three regions with the significant reduction in 5HT contents in the hypothalamus and amygdala, while hippocampus did not show any significant change in 5HT contents when compared with saline treated rats. Other studies have shown that an acute administration of hypericum extract at a dose of (10mg/kg) has caused an increase in 5-HT and 5HIAA in hypothalamus and hippocampus (Yu, 2000) while Kumar and coworkers (Kumar *et al*., 2001), have found decrease in 5-HT and 5HIAA levels in hippocampus & hypothalamic region of the brain, these regions were examined after 30 min of hypericum perforatum administration at the doses of 50 and 200mg/kg, p.o. The discrepancies in these results may be due to the different doses and different time course. In the present study, an increase in 5HIAA in unstressed rats by administration of SJW may be due to the increase in intracellular metabolism of 5HT without its subsequent release, as previous studies on SSRIs have shown that an acute administration of these drugs leads to little or no increase in 5HT levels at postsynaptic sites and potently inhibit 5HT neuronal firing activity. The effect is produced by the activation of somatodendritic 5-HT_{IA} auto receptors, that produces a preferential increase in extra cellular 5HT in mid brain raphe nuclei as compared to terminal projection areas (Adell and Artigas, 1991). According to these findings, it may be suggested that an acute administration of SJW in FST in the present study cause an activation of somatodendritic 5-HT_{IA} auto receptors in the raphe nuclei that result in accumulation of 5-HT & 5HIAA in the terminal projection areas of the limbic brain regions examined. The increase in 5HT in FST by SJW administration in the present study is probably due to the combined influence of stress and SJW administration. It is possible that sustained administration of SJW may increase extra cellular availability of 5HT to similar magnitude in pre and postsynaptic projection areas, as reported previously in experiments done on SSRIs (Bel and Artigas. 1993). The effects of paroxetine, imipramine and desipramine were compared with hypericum, it was found that hypericum at high doses affect the 5HT neuronal uptake mechanisms in a manner more reminiscent of TCAs than SSRIs (Misane and Ogren, 2001). Other findings by Butterweck have shown that long-term administration of hypericin (0.2 mg/kg, p.o) and hypericum extract (500 mg/kg, p.o) increased 5-HT levels in the hypothalamus (Butterweck *et al*., 2002). These authors found that long term but not short-term administration of SJW and its active constituent hypericin modify levels of neurotransmitters involved in the pathophysiology of depression.

The present study shows the decrease in the serum total tryptophan following swim stress. These results are consistent with the previous findings, which have reported that immobilization stress for two hours decreases total tryptophan while increases free tryptophan levels in serum. The increase in free tryptophan pool in plasma may not reflect the concentration of tryptophan increasing in the brain has been suggested by these studies (Kenett *et al*., 1986). On the contrary, other studies have suggested that the foot shock stress cause an increase in 5HT turnover due to increase in plasma tryptophan levels (Dunn, 1988). Tryptophan in the brain is catalyzed by tryptophan hydroxylase to 5 hydroxytryptophan (an intermediate in 5HT synthesis). Tryptophan hydroxylase is highly unsaturated with its amino acid substrate, any increase or decrease in intraneuronal tryptophan levels apparently will cause a parallel changes in enzyme activity. Findings have suggested that tryptophan uptake by the brain does not only depend on the availability of free tryptophan in plasma, however, its ratio with other large neutral amino acids (LNAA) is a main determinant that competes for the same uptake mechanism. Ratio of tryptophan to other LNAA in plasma is known to be affected by food intake (Fernstrom, 1979) and as well as on binding of tryptophan to albumin that has shown to be effected by the number of antidepressants (Badawy *et al*., 1991). Immobilization stress for 30 min has also been determined to change the concentration of various amino acids in plasma. These studies suggested that amino acid concentrations in plasma are the important determinant in stress related pathologies (Milakofsky *et al*., 1985). Later on, foot shock stress was also shown to alter the ratio of tryptophan to other LNAA. It was found that the brain tryptophan was found to increase due to increase in serum tryptophan while concentration of other LNAAs were found to decrease or unchanged (Malyszko *et al*., 1995). Several studies have suggested that the rise in free tryptophan concentration markedly increases the brain tryptophan that is associated with the increase in 5HT turnover (Curzon, 1981; Dunn and Welch, 1991). Keeping in view these studies, the increase in regional brain serotonin metabolism following swim stress may reflect the cerebral accumulation of tryptophan in the present findings. The present study has also determined that an acute administration of SJW in swim stressed rats has shown to decrease serum total tryptophan These findings are in accordance with the studies by (Yu, 2000) have also found reduction in total serum tryptophan levels following administration of hypericum extract. An acute administration of SJW in the present study in rats that were not exposed to swim stress also showed decrease in total tryptophan levels. Considering lower total tryptophan levels in the present findings, it is uncertain to suggest whether the alterations in regional serotonin metabolism is due to the increase in free tryptophan or due to the displacement of bound tryptophan by SJW that compete with other LNAA for cerebral uptake mechanism.

Present study has identified a robust increase in corticosterone when rats were exposed to acute swim stress. Previous studies have reported that the stressful events cause the activation of HPA axis resulting in sustained increase in plasma corticosterone or cortisol. HPA axis hyperactivity is controlled by glucocorticoid receptors in the hypothalamus and hippocampus that maintain the normal level of corticosterone through negative feedback loop. Also, the attenuation of the HPA axis hyperactivity by acute or chronic treatment of various antidepressants has been widely reported (Connor *et al*., 2000). In the present study SJW did not show any reduction in plasma corticosterone levels. The present studies are in agreement with the previous findings have suggested that extracts of SJW LI 160 and two of its active constituents hypericin and hyperforin have significantly increased 5HT mediated corticosterone release (Franklin *et al*., 2000). Other studies (Frost *et al*., 2003) have also found no change in HPA axis function or down regulate circulating levels of plasma corticosterone after chronic treatment of SJW (daily gavage for 8weeks). In contrast, flavonoids extracts of the SJW are well reported for their ability to reduce HPA axis activity (Butterweck *et al*., 2004).
Administration of SJW in unstressed rats in the present study also showed an increase in corticosterone. Several studies have suggested that 5HT1A receptors of the hippocampus are under regulation of adrenal steroids or corticosterone (Chalmers *et al*., 1993) and the removal of glucocorticoid receptors or adrenolectomy may cause a decrease in 5-HT metabolism (Farisse *et al*., 1999). Considering these views, it may be suggested that the increase in 5HT metabolism in the present study is the result of increase in corticosterone that is due to the hyper activation of HPA axis. Therefore, it may be suggested that SJW administration in the present study altered HPA axis activity by modulating serotonergic neuro-transmission function in presence or in absence of stress. SSRIs like paroxetine and venlafexine (SNRIs) have also been reported to fail in reducing FST induced HPA axis hyperactivity (Connor *et al*., 2000) Investigating the mechanism of action of SJW, present study may suggest that SJW affect through serotonergic system and possess mechanism of action similar to SSRIs. Further, its chronic administration on HPA axis function must be studied that would be helpful in understanding the mechanism of its action in depressive therapy.

It was determined in the present study that SJW administration increases brain serotonin content by altering tryptophan levels in the serum. Hyperactivity of HPA axis by SJW administration may determine the activation of hippocampal $5HT1_A$ receptors that is also consistent with the accumulation of 5HT in postsynaptic projections in amygdala, hypothalamus and hippocampus and that may result in inhibition in 5HT neuronal activity and decrease their firing activity. This property of SJW may suggest its anxiolytic property. In conclusion, it may be suggested that SJW act by altering serotonergic neurotransmission, auto receptor activity and HPA axis activity that involve in the pathophysiology of depression. But it is unclear whether SSRIs or NE reuptake blockers would be expected to share the neurochemical response produced by SJW.

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