St. John's Wort increases brain serotonin synthesis by inhibiting hepatic tryptophan 2, 3 dioxygenase activity and its gene expression in stressed rats

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Abstract: We aimed to investigate the effects of herbal St. John's Wort (SJW) on transcriptional regulation of hepatic tryptophan 2, 3 - dioxygenase (TDO) enzyme activity and brain regional serotonin (5-HT) levels in rats exposed to forced swim test (FST). TDO mRNA expression was quantified using real-time reverse transcription polymerase chain (RT-PCR) reaction and brain regional indoleamines were determined by high performance liquid chromatography coupled to fluorescence detector. Behavioral analysis shows significant reduction in immobility time in SJW (500mg/kg/ml) administered rats. It was found that pretreatment of SJW to rats did not prevent stress-induced elevation in plasma corticosterone levels however it increases serotonin synthesis by virtue of inhibiting hepatic TDO enzyme activity and its gene expression, ascertaining the notion that there exists an inverse relationship between hepatic TDO enzyme activity and brain 5-HT. The drug also decreases serotonin turnover in all the brain areas (hypothalamus, hippocampus amygdala) in stressed rats endorsing its monoamine oxidase inhibition property. Inhibition of TDO enzyme activity and its gene expression by the drug provides new insights for the development of therapeutic interventions for stress related mental illnesses.

Keywords: Tryptophan 2, 3 - dioxygenase, serotonin, tryptophan metabolism, TDO mRNA expression, St. John's Wort

INTRODUCTION

Extracts of *Hypericum perforatum*, commonly called St.John's wort, have been used for centuries in herbal medicine and have been clinically studied since the early 1990s (Röder *et al*., 2004). Drugs based on Hypericum extract are widely employed in Europe and are gaining popularity in the United States (Kasper and Dienel, 2002; Lecrubier *et al*., 2002). Hypericum extract contains at least 10 active constituents that may contribute to its pharmacological effects (Wagner and Bladt, 1994). Components known, or suspected, to play a role in antidepressant activity include phloroglucines (e.g. hyperforin), naphthodianthrones (e.g. hypericin) and the flavonoids (e.g. quercitrin). Many beneficial effects of SJW have been reported for stress related illnesses in clinical studies (Volz *et al*., 2002; Kasper *et al*., 2008), and therefore commonly used in many countries to treat various illnesses particularly depression. Recent research suggests the effectiveness of SJW in treating other ailments, including cancer, inflammation-related disorders, eczema, burns, bacterial and viral diseases and as an antioxidant and neuroprotective agent (Klemow, 2011). Studies have demonstrated the alleviative effect of SJW on stress-induced aggravation such as depression condition and cognitive impairment (Butterweck *et al*., 1997; Trofimiuk and Braszko, 2008; Kasper *et al*., 2010; Bukhari and Dar, 2013). Various stress models (See review Jaggi *et al*., 2011) have been developed that are useful for screening anti-depressant and anti-cognitive impairment drugs.

Hepatic tryptophan 2, 3 -dioxygenase (TDO) is the rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism in the periphery, catalyzing the oxidative homeostasis and regulating plasma tryptophan concentrations. The tetrameric enzyme contains two haem units per tetramer that is iron and copper dependent and utilizes molecular oxygen in the catalytic cycle, though multiple tryptophan analogues can activate TDO (Brady *et al*., 1972). Evidences reported, only 1% of dietary tryptophan not utilized for protein synthesis is converted to serotonin while greater than 95% is metabolized in kynurenine pathway (Botting, 1995) The major site of expression of TDO is the liver. Studies using reverse transcriptase PCR (RT-PCR) and immunohistochemical techniques have described TDO mRNA and protein expression in astrocytes within human frontal cortex (Miller *et al*., 2006). Also, recent studies suggest that the enzyme and its activity are up regulated in the anterior cingulated cortex of patients with schizophrenia and bipolar disorder (Miller *et al*., 2006) Hyperactivity of hypothalamic-pituitary adrenal (HPA) axis in depression is associated with high levels of glucocorticoids/ corticosterone (Varghese *et al*., 2001), which is a key factor to induce TDO enzyme beside other factors including substrate (tryptophan) and cofactor (haem) (Welch and Badawy 1980). Corticosterone concentration

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can be regulated by HPA axis feedback loop. Therefore, inhibition in TDO activity is considered as prevention of tryptophan catabolism by the liver. It is clear that peripheral TDO activity is inversely related to brain tryptophan concentration, which is dramatically increased by inhibiting TDO activity (Salter *et al*., 1995). It remains unclear, how important are the moderate changes in peripheral tryptophan concentration that involve in the etiology and pathophysiology of major depression or other stress- related neuropsychiatry disorders. It is clear however that, TDO expression and activity increases by stress-related glucocorticoid hormones via actions at both transcriptional and translational levels (Schimke *et al*., 1965). Previous investigations on alcohol withdrawal syndrome have suggested that the hormonal induction by corticosterone enhances the transcription of tryptophan 2,3 -dioxygenase gene, increases TDO activity and the signs appear during withdrawal syndrome are consistent with its induction (Bano *et al*., 1996). Further, investigations have suggested that cyclohexamide administration inhibits the translational activity of TDO during alcohol withdrawal (Oretti *et al*., 1996).

The findings that antidepressants of different pharmacological profiles such as imipramine, paroxetine, fluoxetine, citalopram, moclobemide, tianeptine, SJW (Badawy *et al*., 1982, Bano *et al*., 2010, Ara and Bano, 2009) share common property of inhibiting TDO activity thereby increasing tryptophan concentrations and serotonin synthesis in the brain. The present study aims to investigate the chronic effects FST on TDO enzyme and its mRNA expression, brain regional serotonin metabolism in SJW pretreated rats.

MATERIALS AND METHODS

Animals and treatment

All animal procedures described below were conducted in strict accordance with the guide for the care and use of laboratory animals (1996). Ethical approval was obtained from institutional animal ethics committee, University of Karachi. Adult male (Albino Wistar) rats (weighing 150 – 200 gm) were used throughout the study. All animals were housed 6 per cage under light and dark conditions at 25±2°C and maintained on free access to standard laboratory diet and tap water under standard housing condition. Rats were divided into two groups (Saline and Drug) containing 12 rats in each. Saline (0.95%) and drug (SJW) treated group of rats were further divided into unstressed and stressed containing 6 in each group. SJW was dissolved in ethanol, 1:3 v/v and was administered orally (oral gavage to stressed and unstressed rats at a dose of 500mg/kg /ml /body weight for 28 days. Animals that were exposed to swim stress procedure were decapitated 5 min after exposure on day 2 of the test following 28 days of drug / vehicle administration. Trunk blood was collected and centrifuged at 4000rpm for 30minutes.The serum was collected and frozen at –70°C until analysis.

Behavioral Analysis

(i) Forced Swim Test (FST)

Animals were exposed to forced swim test (Porsolt *et al*., 1978) as described in detail (Ara and Bano, 2012). Behavior during 5-min test swimming session behavior was scored using a time sampling method This method has previously been described and shown to be reliable and valid for detecting the effects of different antidepressant drugs (Detke *et al.*, 1995). At the end of each 5-s period during the test session, the scorer rated the rat's behavior as one of the following three.. Immobility was scored when the animal was making the minimum movements necessary to stay afloat. Swimming was scored when the animal actively swam around the tank (46 cm high and 20cm inner diameter) filled with 30 cm water (temperature 21±2°C), making movement greater than those necessary to stay afloat. Climbing was scored when the animal made vigorous thrashing movements with its fore paws, usually directed against the sides of the tank. Behavioral activity was videotaped and the results are shown as the total time in seconds for each behavioral category.

(ii) Open Field Test

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. The lines divided the floor into sixteen 18x18 cm squares. Rats were placed into the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, rats were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity (Walsh and Cummins, 1976).

Brain indoleamine analysis

Brain indoleamine analysis was done by using high performance liquid chromatography coupled to fluorescence detector (HPLC-FL). After decapitation, the brains were rapidly removed and hypothalamus, amygdala and hippocampus were isolated. After weighing the respective regions, the tissues were homogenized and deproteinised in volumes of 0.1M Perchloric acid (1g in 4ml 0.1M perchloric acid). The brains were sonicated at 0-4°C at a medium setting for -30 sec period. After adding 0.5ml of 4M perchloric acid and mixing the samples were spun at 10,000g for 10 min and a portion of supernatant was taken and stored at -70°C until analysis. A reverse phase chromatography HPLC-FL was used in analysis of tryptophan (TRP), 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA). The ratio of 5-

HIAA/5-HT was used as an index of 5-HT turnover. For mobile phase 0.01 M sodium acetate was made and pH4.5 was adjusted with glacial acetic acid and finally the volume was made upto 1L with deionized water. After filtering mobile phase, 15% methanol was added and was passed through the Octadecylsilane (ODS) 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 254nm excitation and 360nm excitation (Anderson *et al.*, 1981).

Preparation and quantification of total RNA

Total RNA from liver tissues was extracted by organic extraction method (Chomczynski and Sacchi, 1987). First strand cDNA was synthesized from total RNA by using M-MLV reverse transcriptase (Promega, Cat. # M1701, USA). Briefly, Oligo dt Primer (Invitrogen Cat. # 18418-012, California USA) were used for cDNA synthesis. For real-time reverse transcription PCR (RT-PCR), cDNA was amplified in a thermal cycler (Corbett, Rotor-Gene 3000), using 197ng cDNA and 5µl (10pM/µl) forward (5'CAGGTACAAGGTGTTCGTGG3'), and reverse primers (5'GGACCACAACATCACGTCTC 3') in a reaction volume of 50µl. β-actin cDNA was utilized as a housekeeping gene and amplified analogously using forward (5'ATGGATGACGATATCGCTGC3'), and reverse primers (5'CTTCTGACCCATACCCACCA 3'). Amplification conditions were followed exposure at 95^{o}C for 5min, following the cycling conditions (94^{o}C for 30sec, 59.5^{o}C for 40sec, 72^{o}C for 30 sec) with 40 cycles and finally held at 72^{o}C for 7min and was kept at 4^{o}C. Resulting threshold cycle (Ct) values were recorded for each gene of interest (TDO) as well as for housekeeping gene (β-actin). The difference in the Ct values (ΔCt) was calculated by subtracting the Ct value of gene of interest from house keeping gene. Relative quantification of mRNA expression of TDO and β-actin was determined by calculating relative quantification (R=2-$^{[\Delta Ctsample-\Delta Ctcontrol]}$.

Enzymatic and serum determinations

The tryptophan 2,3-dioxygenase (TDO) activity was determined in rat liver homogenates (made by taking 2 g of perfused frozen liver tissue homogenized in 13ml of 0.14M KCl, pH7 at 0^{o}C with polytron homogenizer spinning at 13000 rpm for 2 to 3 minutes), either in the absence (holo enzyme activity) or in the presence (total enzyme activity) of added haematin 2M (heamatin dissolve in 0.1M NAOH) as previously described in the detail (Bano and Sherkheli, 2003) Corticosterone levels were determined by the standard procedures (Glick *et al.*, 1964).

Drug and chemicals

The plant extract of *Hypericum perforatum* (standardized on 0.3% Hypericin contents) was a gift from Medics Laboratories, Karachi, Pakistan. Haematin hydrochloride and L-tryptophan from Sigma Chemical Co. (St. Louis, Mo). All other chemicals were of highest analytical grade

STATISTICAL ANALYSIS

Statistical analysis was done using 2- way analysis of variance (ANOVA) or where appropriate student's test. The data, presented as means ± SD (n=6) for each data point. P values less then 0.05 were considered significant.

RESULTS

Fig. 1 shows effects of the chronic administration of SJW on behavior in FST. The SJW showed significant effects on behavior of rats. Data analyzed by students t-test showed that floating time was decreased by 79%; $p<0.001$ while there was increase in swimming time by 24.12%; $p < 0.001$ and climbing time by 193%; $p<0.001$.

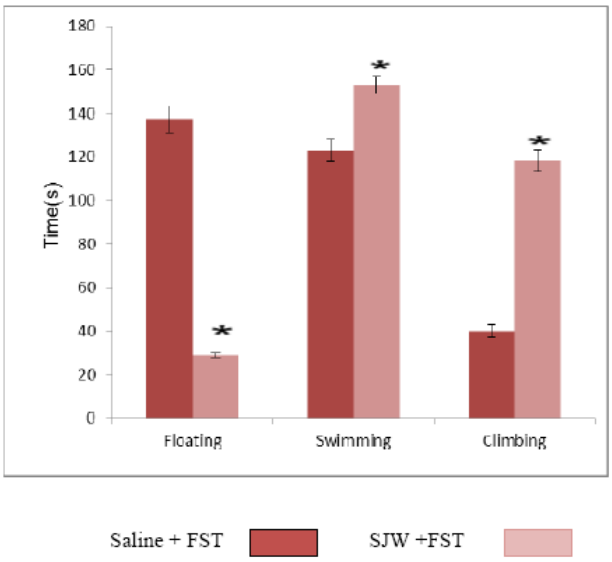

Fig. 1: Effects of chronic administration of SJW on behavior of rats in FST All values, presented as means± SEM of six rats, statistical analysis was performed using student's t-test. The significance of difference is indicated by *P<0.001 when SJW treated stressed group was compared with respective stressed controls.

In open field test SJW did not cause any effect on locomotor activity (number of entries in the centre, time spent in the centre, ambulatory, grooming activities and rearing count) as compared to saline controls (data not shown).

Table 1 shows the effects of FST on saline injected and SJW injected rats on serum TRP and corticosterone

levels. Data analyzed by 2-way ANOVA in the table shows significant effect of stress on serum TRP (F=15.87; p<0.01) while insignificant change corticosterone was observed. The effects of SJW was significant on serum TRP (F=79.7; p<0.01) and corticosterone (F=12.69; p<0.01). Interaction between FST X drug showed insignificant effect on serum TRP while significant effect on corticosterone levels (F=43.73; p<0.01).

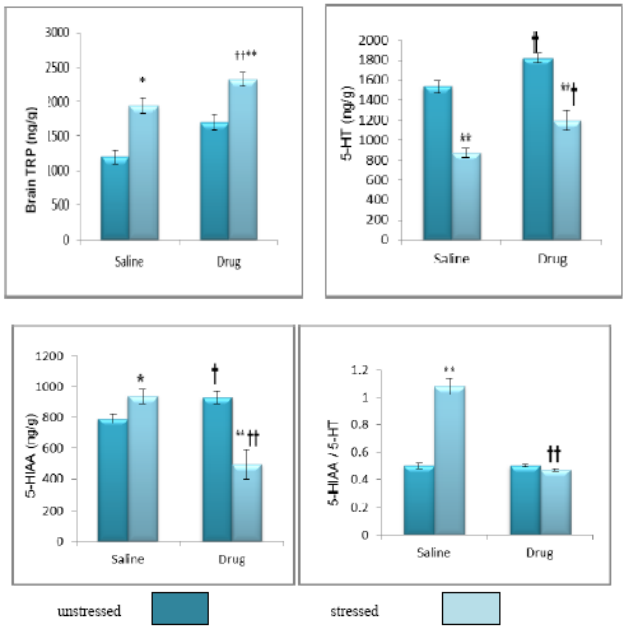

Fig. 2: Effects of chronic administration of SJW on brain regional (Hypothalamus) indoleamines level in rats. Experimental details are given in material methods section. All values, presented as means ± SEM of six rats, statistical analysis was performed using two ways ANOVA followed by Newman-Keul's test. The significance of difference is indicated by *p<0.05 & **p<0.01 when stressed group was compared with respective unstressed group. And, †p<0.05 & ††P<0.01 when SJW injected group was compared with similarly treated saline group

Table 2 shows the effects of FST on saline injected and SJW injected rats on hepatic TDO enzyme activity. Data analyzed by 2-way ANOVA shows significant effect of stress on holo (F=89.0, p<0.01) apo (F=35.45; p<0.01) and total enzyme activities (F=168.2.; P<0.01). The effects of drug SJW caused insignificant effect on holo-enzyme, while caused significant effect on apo-enzyme (F=109.9; p<0.01) and total- enzyme (F=110.6; p<0.01). Interaction between FST X Drug showed significant effect on holo-enzyme F=8.67, p<0.01 apo-enzyme F= 18.20; p<0.01 and total F=37.5; p<0.01 enzyme activities.

Figs. 2-4 show the effects of chronic administration of SJW on regional brain indoleamines. Data analyzed by 2 way ANOVA showed significant effects of FST on brain TRP in hypothalamus (F=13.89; p<0.05) and in amygdala (F=5.98; p<0.05). Effects of drug was significant in hypothalamus (F=23.0; p<0.01), amygdala (F=26.4; p<0.01) and in hippocampus (F=11.4; p<0.01). FST X drug interaction was insignificant in amygdala regions, however, significant in hypothalamus (F=16.0; p<0.01) and in hippocampus (F=5.2; p<0.05). Significant effects of stress on 5-HT were seen in all three regions in hypothalamus (F=46.86; p<0.01) in hippocampus (F= 5.70; p<0.05) and in amygdala (F=10.54; p<0.01). Similarly, effect of stress on 5-HIAA levels showed significant effect only in hypothalamus (F=10.50; p<0.0). Stress also caused significant effect on 5-HIAA/5-HT turnover in all the three regions (hypothalamus F=26.51; p< 0.01, amygdala F=8.40; p<0.01, and in hippocampus 12.83; p<0.01). Effects of drug on 5-HT concentrations in hypothalamus (F=15.43; p<0.01) amygdala (F=8.90; p<0.01) and in hippocampus (F=14.71; p<0.01) were significant. Similarly, effect of drug on 5-HIAA levels in hypothalamus F=11.63; p<0.01. SJW also caused significant effect on 5-HIAA/5-HT turnover in all the three regions i-e hypothalamus (F=32.33; p<0.01) amygdala (F=10.13; p<0.01) and in hippocampus (F= 29.38; p<0.01). Stress X drug interaction showed insignificant effect on 5-HT in hypothalamus, amygdala and in hippocampus but caused significant effects on 5-HIAA in hypothalamus F=37.85; p<0.01 and in hippocampus F=11.13; p<0.01. FST x drug interaction also caused significant effect in hypothalamus F=31.58; p<0.01 and in hippocampus F=13.58; p<0.01 on 5-HIAA/5-HT turnover.

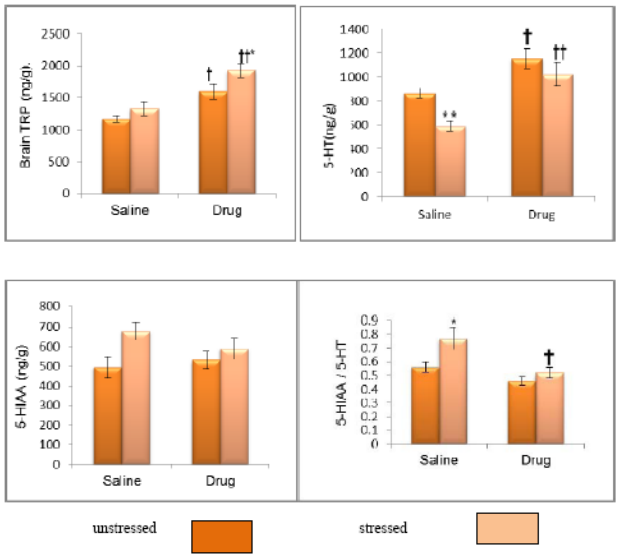

Fig. 3: Effects of chronic administration of SJW on brain regional (amygdala) indoleamines level in rats. Experimental details are given in material methods section. All values, presented as means ± SEM of six rats, statistical analysis was performed using two ways ANOVA followed by Newman-Keul's test. The

significance of difference is indicated by *p<0.05 & **p<0.01 when stressed group was compared with respective unstressed group. And, †p<0.05 & ††P<0.01 when SJW injected group was compared with similarly treated saline group

Fig. 5 shows the effects of chronic administration of SJW on TDO mRNA expression when. Data was analyzed by 2-way ANOVA. Effects of stress (F=54.81; p<0.01) and drug (F=16.38 p<0.01) were significant however, FST x Drug interaction was not.

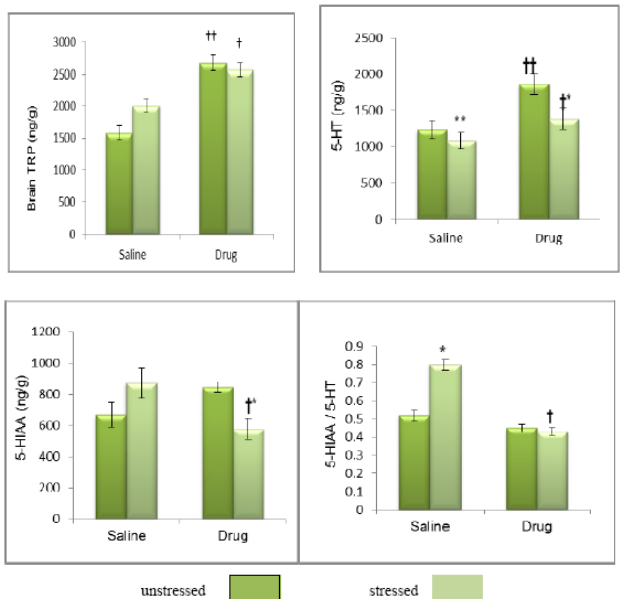

Fig. 4: Effects of chronic administration of SJW on brain regional (hippocampus) indoleamines level in rats. Experimental details are given in material methods section. All values, presented as means ±SEM of six rats, statistical analysis was performed using two ways ANOVA followed by Newman-Keul's test. The significance of difference is indicated by *p<0.05 & **p<0.01 when stressed group was compared with respective unstressed group. And, †p<0.05 & ††P<0.01 when SJW injected group was compared with similarly treated saline group

DISCUSSION

The present investigation shows decrease in immobility time in FST in SJW administered rats. These findings are in agreement with several previous investigations (Butterweck, 2003a; Butterweck *et al*., 2003b). Detke *et al*. (1995) have proposed that all antidepressants exhibit similar property of reducing immobility time in FST but they are discriminated on the basis of swimming and climbing behavior, depending upon their mechanism of action. And, the time spent in swimming and climbing behavior is interpreted as serotonergic and noradrenergic properties of antidepressant compounds (Cryan *et al*., 2005; Detke *et al*., 1995). Thus, behavioral effects in FST by the chronic administration of SJW are worth mentioning showing characteristic similar to noradrenergic and serotonergic antidepressants. Though, some acutely administered non-antidepressant drugs such as psycho-stimulants (caffeine and amphetamines), cholinergics and convulsants are known to reduce immobility time in FST, as they tend to increase motor activity (Browne, 1979; Betin *et al*., 1982). Therefore open field test is used in order to neglect false positive effects on motor activity of rodents in FST produced by administrating the drug. We demonstrated that chronic administration of SJW at the doses used in the present findings did not interfere with the ambulatory activity of rodents rats.

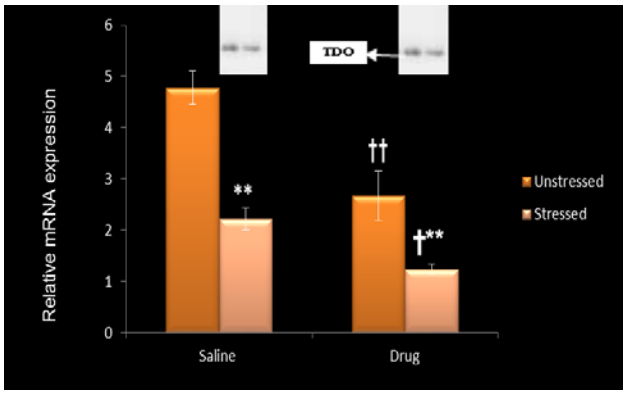

Fig. 5: Effects of Chronic Administration of SJW On Hepatic TDO mRNA Expression In Rats (gel pictures shows relative expression of TDO mRNA expression). Experimental details are given in material and methods section. All values, presented as means ± SEM of six rats, statistical analysis was performed using two ways ANOVA followed by Newman- Keul's test. The significance of difference is indicated by *p<0.05 & **p<0.01 when stressed group was compared with respective unstressed group. And, †p<0.05 & ††P<0.01 when SJW injected group was compared with similarly treated saline group

Serotonin controls the stress response by interacting with the hormonal HPA-axis and neuronal sympathetic nervous system (SNS). Tryptophan hydroxylase is the rate-limiting enzyme in 5-HT biosynthesis, and the recent identification of a second, neuron-specific tryptophan hydroxylase-2 isoform opened up a new area of research. It has been proposed (Chen and Miller, 2012) that upon activation of adrenal cortisol secretion, the cortisol surge induces tryptophan hydroxylase-2 expression and de novo 5-HT synthesis then the induced 5-HT in turn inhibits cortisol secretion by modulating the adrenal sensitivity to ACTH via the suprachiasmatic nuclei (SCN)-SNS-adrenal system, such that it contributes to the feedback inhibition of cortisol production. We have found that FST increases 5-HT turnover but increase in brain tryptophan were significant only in hypothalamus.

Table 1: effects of SJW administration on serum TRP & corticosterone concentrations in rats

Enzyme activity (µmoles of kynurenine formed/hr/g wet wt. of liver)	Saline		Drug		Two Way ANOVA (df 1,20)		
	Unstressed	Stressed	Unstressed	Stressed	FST	Drug	FST×Drug
Serum TRP (µg/ml)	22.49±1.57	17.0± 0.0.95**	11.6± 1.0††	8.59± 0.61*††	F=15.87 p<0.01	F=79.71 p<0.01	F=1.02 NS
Corticosterone (µg/dl)	41.99±2.41	60.25± 1.54**	80.52± 3.88††	75.35± 3.28††	F=5.05 P<0.05	F=84.76.6 p<0.01	F=16.17 p<0.01

Table 2: effects of SJW administration on hepatic TDO enzyme activity in rats

Enzyme activity (µmol of Kynurenine formed/hr/gm wet wt. of liver)	Saline		Drug		Two Way Anova (df 1,20)		
	Unstressed	Stressed	Unstressed	Stressed	FST	Drug	FST×Drug
Holo-enzyme	2.29±0.07	1.05± 0.12**	1.81± 0.10††	1.17± 0.07	F=89.0 P<0.01	F=3.15 NS	F=8.67 P<0.01
Apo-enzyme	2.58±0.15	1.52± 0.07**	1.05± 0.09††	0.88± 0.07††	F=35.45 p<0.01	F=109.9 p<0.01	F=18.20 P<0.01
Total-enzyme	4.87±0.16	2.58± 0.07**	2.87± 0.14††	2.06± 0.05**††	F=168.2 p<0.01	F=110.6 p<0.01	F=37.5 P<0.01

Experimental details are given in material methods section. All values, presented as means ±SEM of six rats, statistical analysis was performed using two ways ANOVA followed by Newman- Keul's test. The significance of difference is indicated by *p<0.05 & **p<0.01 when stressed group was compared with respective unstressed group. And, †p<0.05 & ††P<0.01 when SJW injected group was compared with similarly treated saline group

We have found that both swim stress and SJW administration to rats increase circulating corticosterone levels. Further pretreatment of SJW did not prevent the stress-induced elevation in plasma hormone levels. SJW, therefore, may not be effective antidepressant drug for depressive disorders that share features with chronic stress and activation of the HPA axis. A similar result was observed for zimelidine and fluoxetine, two specific serotonin reuptake inhibitors (SSRIs) (Lopez *et al*., 1998). It could be inferred that SJW might have an influence on the serotonergic system comparable to that of the SSRIs. There is evidence from the literature that different compounds from Hypericum Perforatum differentially affect neurotransmitter systems (Butterweck *et al*., 2001).

Present study showed decreased 5HIAA/5-HT ratio in sub-cortical regions in SJW administered stressed rats confirms that SJW inhibits monoamine oxidase and facilitate 5-HT neurotransmission in limbic brain areas in stress. Similarly, antidepressants such as TCAs desipramine, SSRIs paroxetine, SNRI's venlafaxine, have been shown to attenuate FST-induced increased serotonergic turnover (Connor *et al*., 2000) that show resemblance in the mechanism of action with herbal SJW. It is considered that forced locomotion is associated with the increase in 5-HT release that depends upon the intensity of stress, its time duration, training procedures and the brain region analyzed (Kirby at al., 1997). Thus, SJW accelerates serotonin synthesis and release that is reflected by increased swimming time during FST. Chronic administration of SJW contributed to increase the concentration of TRP in the brain regions in stressed and unstressed rats probably via increasing its uptake to the brain and increased the rate of serotonin synthesis. Earlier we have reported (Bano *et al*., 2010) that acute SJW (10mg/kg) administration increases tryptophan availability to the brain secondarily to inhibition of TDO enzyme activity. It is worth mentioning that the rate of serotonin synthesis increases by the efficiency of TRP hydroxylase that remains half saturated by its substrate under normal physiological conditions. Any increase or decrease in intraneuronal tryptophan levels will cause parallel changes in enzyme activity. These observations are in accordance with the findings reported earlier that chronic sound stress increases midbrain TRP hydroxylase enzyme activity considerably via initializing new protein synthesis (Azmitia and McEwen, 1997). Also, long-term treatment by SJW (500mg/kg) increased 5-HT contents in the hypothalamus and hippocampus regions (Butterweck *et al* 2002). These observations are in line with our present findings showing that the chronic administration of SJW appeared to enhance 5-HT synthesis in the hypothalamus, amygdala and hippocampus in swim stressed rats and in all the regions in unstressed rats. Further, consistent reduction in 5-HIAA by the repeated administration of SJW both in stressed and unstressed rats may reflect release of serotonin to facilitate its neurotransmission and bring into line the mechanism of action of SJW as serotonergic reuptake blocker. High doses of SJW have been demonstrated to act as monoamine oxidase inhibitor (Misana and Ogren, 2001). However, these effects have not been observed with the

consumption of SJW at dosages recommended for the treatment of depression. Taken into account these observations the characteristic of SJW can be speculated as classical SSRIs. *Hypericum perforatum* extracts are known to inhibit the synaptosomal uptake of serotonin, dopamine, noradrenaline, Gamma-aminobutyric acid with similar potency (Misana and Ogren, 2001). It is note worthy that chronic administration of SJW reduced stress-induced increased corticosterone level, which is in accordance with several previous findings (Frost *et al*., 2003; Butterweck *et al*., 2004). In view of these facts and findings, SJW (chronically) may directly or indirectly via corticosterone increased free TRP uptake to the brain (in brain regions examined in the present findings) that might result in lowering of plasma total TRP levels in stressed and unstressed rats. In addition, mechanism of action of SJW to increase brain TRP for serotonin synthesis has reciprocal interaction with hepatic TDO enzyme. As shown in present findings, chronic administration of SJW appeared to inhibit TDO enzyme activity in stressed rats that is in accordance with the inhibition in TDO mRNA expression. These results could be explained by the reduction in FST-induced increased corticosterone levels by the chronic administration of SJW that rendered to inhibit synthesis of apo-enzyme as reflected by the inhibition in mRNA expression.

In addition, there exists another mechanism (Badawy *et al*., 1982) by which SJW might have interrupted conjugation of apo-enzyme with heme and that reflects the mechanism of action of SJW similar to other SSRIs, as reported earlier (Bano *et al*., 2010; Ara and Bano, 2012). Accordingly inhibition in TDO mRNA expression was seen in SJW-administered unstressed rats that support our previous findings that show inhibition in TDO enzyme activity by preventing conjugation of apo-enzyme with heme (Badawy *et al*., 1977, 1982; Bano *et al*, 2010). It seems likely that the drug allows the utilization of TRP by the body to compensate 5-HT deficiency in the brain and non-utilization of heme by the TDO due to inhibition in apo-heme conjugation by the drug that may lead to repress its own synthesis in the liver by feed back mechanism.

CONCLUSION

It is concluded that full understanding of the mechanism of action of SJW in stress related mental illness will require an integrative approach that also considers peripheral tryptophan metabolism. Inhibition of TDO enzyme gene expression by the drug provides new insights for the development of therapeutic interventions for stress related mental illnesses.

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