SEROTONERGIC MEDIATION EFFECTS OF ST JOHN'S WORT IN RATS SUBJECTED TO SWIM STRESS

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ABSTRACT

Present study shows the effects of St John's Wort (SJW) (20mg/kg) on swim stress induced changes in tryptophan (TRP) metabolism and disposition in rats. The results show that after forced swim test (FST) hepatic tryptophan pyrrolase (holo and total) activities were significantly decreased ($P<0.001$). Liver TRP was increased ($P<0.001$) while serum TRP was decreased ($P<0.01$). Brain TRP, 5-hydroxytryptamine (5-HT, Serotonin) and 5-hydroxyindole acetic acid (5-HIAA) concentrations were increased ($P<0.001$), similarly immobility time during swim test was also increased. SJW pretreated FS group of rats showed reduced holo enzyme activity ($P<0.001$) while increase in total and apo enzyme activities ($P<0.001$). There was significant decrease in liver TRP ($P<0.01$), serum TRP ($P<0.05$), brain TRP ($P<0.001$), 5-HT ($P<0.001$) and 5-HIAA ($P<0.001$) concentrations with reduction in immobility time during swim test when compared with saline injected FS group. SJW injected group but when compared with untreated controls showed significant increase in total and apo enzyme activities ($P<0.001$) while holo enzyme activity was decreased ($P<0.001$), serum TRP, brain TRP and 5-HIAA levels were significantly decreased ($P<0.001$). Changes in 5-HT concentrations were not significant. It is concluded that SJW treatment alter stress induced augmented 5-HT levels by decreasing precursor availability to the brain and that serotonergic system is involve in the mechanism of action of the drug.

Keywords: St. John's Wort, Swim-stress, 5-HT, behavior, tryptophan.

INTRODUCTION

Tryptophan pyrrolase (2-tryptophan 2,3 dioxygenase EC.13.11.11), a metalloprotein containing porphyrin rings is the first and rate limiting enzyme for hepatic tryptophan (TRP) metabolism via kynurenine nicotinamide pathway in liver and therefore plays a key role in regulating flux of TRP in to relevant metabolic pathways (Ren & Correia. 2000), such as synthesis of neurotransmitter serotonin in the brain . The enzyme is composed of four identical sub units and in its fully assembled tetrameric forms requires 2 molecules of haem per molecule of enzyme protein for functional competence. The active reduced form of the enzyme (holo enzyme) does not need the addition of cofactor haematin, whereas, the inducible form apoenzyme requires the addition of haematin for its activity. The enzyme is induced by its substrate (TRP) and glucocorticoids (Badawy, 1977). Increased catalytic activity of tryptophan pyrrolase contributes to the lower plasma tryptophan concentrations in major depression. Depletion of tryptophan plays key role for deficient 5-hydroxytryptamine (5-HT) in the etiology of depression (Smith *et al*., 1997). There are evidences that in depressed patient reduction in plasma tryptophan and its availability may contribute to abnormalities in brain 5-HT function. The biogenic amine theory of depression suggest it is caused by a deficiency of serotonin or epinephrine, These neurotransmitters are actively secreted into synapses by neurons then are taken up by receptors at the post synaptic neurons. They are subsequently either stored or catabolized by monoamine oxidase. Therefore, substances having a positive effect on depression (drugs or phytochemicals) should impact the levels of these neurotransmitters by increasing biogenic amine synthesis by (1) increasing biogenic amine synthesis (2) decreasing their catabolism by inhibiting monoamine oxidase (3) inhibiting their reuptake Antidepressants are thought to normalize the disturbances in monoamine function that occur in affective disorders (Frazer, 1997).

Deprisin a film coated herbal tablet for oral use containing 450 mg of standard extract of the plant Hypericum perforatum (popularly known as St. John's Wort (SJW) has been used in folk medicine for thousand of years and has most recently been proved scientifically as an effective treatment for mild to moderate depression .Studies have shown SJW exhibits antidepressant properties in human (Linde *et al*., 1996; Volz, 1997; Nathan, 1999) and exerts antidepressant like effect in rodents in experimental animal paradigms (Butterweck *et al*., 1997; Butterweck 1998; Ozturk, 1997) such as the forced swim test (FST) (Porsolt *et al*., 1977; Borsini & Meli, 1988; Willner, 1991). In one study it was reported that acute administration of 50 to 200mg/kg Indian Hypericum Perforatum significantly decreases the levels of 5-HT and its turnover in contrast augments the levels of norepinephrine and its turnover in various brain regions (Kumar *et al*., 2001) SJW enhances the concentrations of

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dopamine, (DA), norepinephrine, NE, serotonin (5-HT) and glutamate in the locus coeruleus of rats (Philippu, 2001). SJW at a dose of 10mg/kg have found to increase 5-HT and 5-HIAA in mouse brain regions with reduction in plasma tryptophan levels. The authors reported that effects of SJW are different from classical 5-HT reuptake blockers (Yu, 2000; Hirano *et al*., 2004).

FST is animal model of depression in the rodent. The forced swim test is used for screening antidepressant activity, potential drugs administered between pretest and test session decrease the duration of behavioural immobility. Effect of FST in different brain regions, have been shown to produce distinct, regionally specific changes in extra cellular 5-HT, an increased of 5-HT in the striatum, a decrease of 5-HT in the amygdala and lateral septum and no change in 5-HT in the frontal cortex or hippocampus (Kirby *et al*., 1995). Kelliher *et al*., 2000 have studied varying the effects of response to the rat forced swim test under diurnal and nocturnal conditions. There is a link between dysfunction in monoamine transmission; that is 5-HT, NE and depression (Delgado *et al*., 1994; Miller *et al*., 1996). It is established that reduced serotonin concentration produces depression, where as its stimulation results in an anxiogenic effect (Chopin and Briely, 1987). The aim of the present study is to examine effects of SJW administration on tryptophan metabolism and disposition in forced swim rats.

MATERIALS AND METHODS

Animals and Treatment

Locally bred male Albino Wistar rats (150-200 gm body wt.) were housed five rats per cage under natural light dark cycle at 25^{o}C ± 2^{o}C room temperature and were maintained on lab chow and water *ad libitum*. Rats were divided into three groups, each group had five rats. The 1st group of rats (untreated control) remained in their home cage. The second group was injected (i.p.) saline (0.95%) (2ml/kg) and then subjected to forced swimming test. The 3rd group of rats were injected St. John's Wort (20 mg /kg) dissolved in vehicle (DMF: Saline) (1:3 v/v) and after 3.5hr were subjected to FST. After these treatments, animals were killed by decapitation at specific time intervals. Brain was taken out within 30 second of the death of animals. Brains, serum and perfused livers were stored at -70^{o}C until analysis.

Forced Swim Test (FST)

Animals were subjected to forced swim test to create a model of depression. Animals were placed in a cylindrical glass tank (46cm tall x 20cm in diameter) of 21^{o}C to 22 ^{o}C, water filled to a depth of 30cm. The water depth of 30cm allowed the rats to swim or float without hind limbs touching the bottom of the tank. Rats were individually placed in the water tank for 15 min pretest. Upon removal, animals were towel dried before returning to their cage. Twenty-four hours later rats were injected saline or SJW. 3.5 hrs after injection, rats were placed once more in the tank for a 5-min test swim. Control animals (untreated) were not subjected to force swimming on either day (Porsolt *et al.*, 1977; Lucki *et al*., 2001).

Behavioral analysis

Behavior during the 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 minimum 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.

Enzymatic and other determination

The tryptophan pyrrolase was determined in liver homogenates (made by taking 2 g of perfused frozen liver tissue homogenized in 13 ml of 0.14 M KCl, PH 7 at 0°C with polytron homogenizer spinning at 1300 rpm for 2 to 3 minutes) either in the absence (holo enzyme activity) or in the presence (total enzyme activity) of added haematin 2 μM (haematin dissolve in 0.1 M NaOH) as previously described in detail (Bano and Sherkheli, 2003). Other parameters of tryptophan metabolism and disposition such as brain tryptophan, 5-HT and its major metabolite 5-HIAA were determined by established spectrofluorimetric procedures (Bano *et al*., 1996).

Chemicals and drugs

St. John's Wort (Deprisin) was a gift from Medics Laboratories Pakistan. Haematin hydrochloride and L-tryptophan were purchased from Sigma chemical Co. St. Louis, MO. All others chemicals were of highest purity analytical grade.

STATISTICAL ANALYSIS

Data was analyzed by student's *t*-test.

RESULTS

Table 1 shows significant inhibition in holo (30.8%; P<0.001) and total (20.4%; P<0.001) enzyme activities after force swim test when compared with untreated control rats. There was significant increase (46%; P<0.001) in total and (127%; P<0.001) apo enzyme activities while holo enzyme activity was reduced by 49.3% (P<0.001) in drug treated FS rats when compared with saline injected controls. Holo enzyme activity was decreased by 64.9% (P<0.001) while total and apo enzyme activities were increased by 16.1% (P<0.001) and

Table 1: Effects of forced swim and SJW (20mg/kg) on basal hepatic tryptophan pyrrolase activity

(μmoles of Kynurenine formed /h/g wet wt.)	Untreated control	Saline + Force swim	SJW + Force swim
Holo enzyme	2.17±0.1	1.5 ± 0.08† -30.8%	0.76 ± 0.06*δ -49.3%* -64.9%δ
Total enzyme	4.15±0.06	3.3 ± 0.09† -20.4%	4.82 ± 0.04*δ +46%* +16.1%δ
Apo enzyme	2.0±0.07	1.8 ± 0.13	4.1 ± 0.10*δ +127%* +105%δ

All values are mean ± SEM for each group (n=5) animals. Significance of difference, saline treated FS group vs. untreated control is indicated by †P<0.001. SJW treated FS vs. saline treated control is indicated by *P<0.001 and vs. untreated control is indicated by δ P<0.001.

Table 2: Effects of force swim test and SJW (20mg/kg) on rat serum total and liver TRP concentrations

Parameters	Untreated control	Saline + Force swim	SJW Wort +Force swim
Serum Total TRP μg/ml	15.11 ± 1.65	7.17 ± 0.33† -52.5%	5.6 ± 0.31*δ -21.8%* -62.9% δ
Liver TRP μg/g	7.8±0.3	11.73 ± 0.37†† 50.3%	9.2 ± 0.63** -21.5%

All values are mean ± SEM for each group (n = 5) animals. Significance of difference, saline treated FS group vs. untreated control is indicated by †P<0.01, ††P<0.001. SJW treated FS group vs. saline treated control is indicated by *P<0.05, **P<0.01 and vs. untreated control is indicated by δP<0.001.

Table 3: Effects of force swim test and SJW (20mg/kg) on brain indole concentrations

Brain indoles (μg/g)	Untreated control	Saline + Force swim	SJW + Force swim
Brain Trp	1.2±0.1	1.9 ±0.07† 58.3%	0.9±0.03*δ -52%* -45%δ
Brain 5-HT	0.6±0.04	1.0 ±0.05† 66.6%	0.54±0.07* -46%
Brain 5-HIAA	0.25±0.015	0.54±0.04† 80%	0.17±0.006*δδ -62.2%* -32%δδ

All values are mean ± SEM for each group (n = 5) animals. Significance of difference, when saline treated FS group vs untreated control is indicated by †P<0.001. SJW treated FS group vs saline treated control is indicated by *P<0.001 and vs untreated control is indicated by δP<0.05, and δδP<0.001.

105% (P<0.001) respectively when drug treated FS rats were compared with untreated control rats.

Table 2 shows serum total TRP concentrations were significantly decreased by 52% (P<0.01) in saline injected FS group when compared with untreated control rats There was significant decrease by 21% (P<0.05) in drug injected FS group when compared with saline injected controls. Table 2 also shows hepatic TRP concentrations were significantly increased (50.3%; P<0.001) in saline injected FS rats when compared with untreated control rats. Table 3 shows that brain TRP, 5-HT and 5-HIAA concentrations were significantly increased by 58.3% (P<0.001), 66.6% (P<0.001) and 80% (P<0.001) respectively in forced swim rats when compared with untreated control rats. While brain TRP, 5-HT and 5-HIAA concentrations were significantly decreased by 52 %(P<0.001), 46% (P<0.001) and 62.2% (P<0.001)

respectively in drug injected FS rats when compared with saline injected controls. Brain TRP and 5-HIAA concentrations were significantly decreased by 25% (P<0.05) and 32 %(P<0.001) respectively in drug injected force swim rats when compared with untreated control rats.

Fig. 1 shows that SJW significantly increased climbing activity by six fold (632%, P<0.001) and decreased floating behavior by 91.3 % (P<0.001) when compared with saline injected controls.

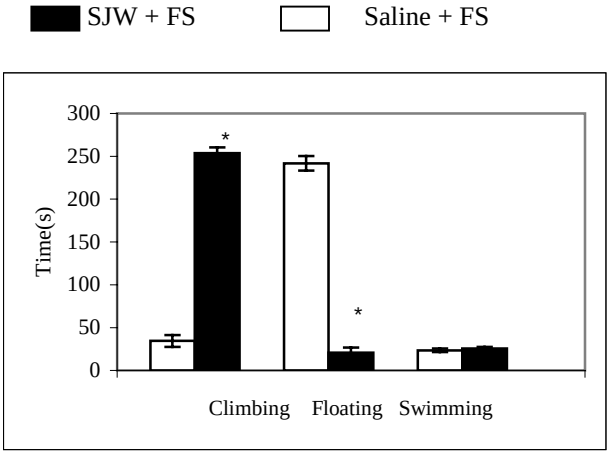

Fig. 1: Effects of acute administration of SJW (20mg/kg) on floating, climbing and swimming in rats. Rats subjected to FST received an acute administration of SJW and control rats received an equal volume of saline. All values are mean ± SEM of five rats. The significance of difference is indicated by ***P<0.001.

DISCUSSION

The effects of forced swim test on tryptophan metabolism and disposition is studied for the first time in rats .We have found that forced swim (FS) stress in rats inhibits hepatic tryptophan pyrrolase activity and increases brain 5-HT concentrations by increasing availability of tryptophan to the brain. Inhibition of hepatic tryptophan pyrrolase may be due to inhibition of conjugation of apoenzyme with haem. An inverse relationship between liver tryptophan pyrrolase activity and brain 5-HT synthesis has been documented under many circumstances (Litman and Correia 1985; Badawy and Morgan 1991; Oretti *et al*., 1996). The mechanism by which tryptophan pyrrolase activity could regulate brain 5-HT synthesis involves a number of factors (a) an altered availability of circulating tryptophan to the brain, whether the pyrrolase activity is decreased or increased (b) direct inhibition of the cerebral uptake of tryptophan by metabolites, specially kynurenine, which are increased after pyrrolase enhancement (Curzon, 1969). (c) Decreased availability of pyridoxal phosphate for 5-hydroxytryptophan decarboxylase in brain because of increased hepatic demand for coenzyme in reactions along the kynurenine pathway following pyrrolase enhancement (Rose and Braidman, 1970). Increased brain serotonin levels improve the ability to cope with stress, while a decline in serotonin activity is associated with depression. Tryptophan is the amino acid that increases serotonin levels in the brain. Changes in brain tryptophan levels is parallel changes in the rates at which serotonin is synthesized in, and released from raphe nucleus neurons. The enzyme tryptophan hydroxylase, which catalyzes tryptophan conversion to 5-hdroxytryptophan (the intermediate in serotonin synthesis), is highly unsaturated with its amino acid substrate, hence any increase or decrease in intraneuronal tryptophan levels apparently will cause a parallel change in the enzyme's net activity. It is not compellingly demonstrated that serotonin-producing nerve terminals have a special uptake mechanism for the amino acid. Its transportation into the brain is dependent on other amino acids with neutral charge .This influences tryptophan exchange via the ratio of tryptophan to the sum of other large neutral amino acids (LNAAs) that share the same brain uptake system (Fernstrom 1983). Later the same group Madras *et al.*, (1973) and Madras *et al*., (1974) in a study on the effects of carbohydrate diet reported that concentration of unbound or free tryptophan in the serum does not predict the changes in brain tryptophan caused by such physiological inputs as eating. Free tryptophan might be correlated with brain tryptophan after certain treatments such as drug administration (Tagliamonte, 1971) or a prolonged fasting period (Knott and Curzon, 1972). In present study, changes in serum total tryptophan are not reflected in brain tryptophan levels after acute forced swim stress, but one cannot exclude the possibility that free fraction might have increased. This notion is supported by finding in which it was reported that after 2.5 hrs immobilization stress in rats, the total tryptophan concentration was decreased (Joseph *et al*., 1984). Later Kennett *et al*., (1986) suggested that rise in level of plasma free tryptophan during immobilization is not responsible for increase in level of brain tryptophan. Although the precise relation ship between plasma and brain tryptophan are controversial, there is evidence that the pool of free (i.e., unbound) tryptophan is the most important (Curzon *et al*., 1979) The entry of free tryptophan to the brain depends on the tryptophan blood concentration as well its ratio concentration to that of other LNAA which competes for brain access (Cryan and Leonard, 2000). It is interesting that catecholamines released from the adrenal medulla during stress apparently have very little effect, because adrenalectomy did not alter stress-related increase in brain tryptophan (Curzon *et al*., 1972; Dunn, 1988).

We have also found increased concentration of brain serotonin and its chief metabolite 5-HIAA and increased

immobility in swim stressed rats. Increase in brain tryptophan appears to be necessary to sustain the increased serotonin catabolism to 5-HIAA that occurs in stressed animals and which may reflect increased serotonin release. Similar increases have been reported previously by Haleem *et al*., (1988) in 2.5hrs immobilized rats. Kirby and coworkers (1995) have found that FST produced region specific changes in extra cellular 5-HT an increase of 5-HT in the striatum, a decrease in 5-HT in the amygdala and lateral septum and no change in 5-HT in the frontal cortex or hippocampus.

Although all antidepressants (ADs) reduced behavioral immobility, those ADs that increase serotonergic neurotransmission predominantly increase swimming behavior where as those that increase catacholaminergic neurotransmission also increase climbing behavior (Cryan *et al*., 2005). The present study shows that single administration of St. John's Wort (20 mg/kg) reduces the immobility time or freezing behavior of rats in the FST when compared with saline injected FST rats. These results are consistent with several other studies (Hirano *et a*l., 2004; Bach-Rojecky *et al*., 2004) that demonstrate that single doses of extract of SJW reduced immobility. Calapi *et al* (2001) have reported that at a dose (250-500 mg/kg) SJW enhanced serotonin, NE and DA content in brain and reduced immobility time in rats. In contrast, we have found low serotonin level and reduced immobility in forced swim rats following SJW treatment, this difference may be due to the higher dose or route of administration of drug.

Liver tryptophan pyrrolase is enhanced by corticosterone-mediated hormonal induction mechanism. Glucocorticoids are known to enhance the transcription of tryptophan pyrrolase gene (Delap and Feigelson, 1978; Nakamura *et al*., 1987). Studies in human and animals have shown increased in plasma cortisol/ corticosteroid following treatment with SJW (Franklin and Cowen, 2001; Franklin *et al*., 1999; Schüle *et al.,* 2001). It is therefore suggested that one fold increase in apoenzyme is may be due to hormonal induction by glucocorticoids after forced swim stress in SJW pretreated rats. In present study St. John's Wort administration at the dose of 20mg /kg shows activation of total and apo enzyme activities with significant decrease in holoenzyme levels. Induction of enzyme activities is confirmed by low level of liver TRP. Generally, TRP cause an initial increase in the haem saturation of the apo enzyme, where as cortisol does not (Badawy, 1977). We have found that haem saturation (expressed as percentage 100-x holoenzyme activity /total enzyme activity) is decreased to 16% where as in saline control it is 45% (not shown in Table1). Early studies have shown that hormonal induction of pyrrolase activity is not associated with increased saturation of the enzyme with its haem cofactor, unlike substrate and cofactor-type activation (Badawy and Evans, 1975). Augmented brain 5-HT and 5-HIAA in FST group are normalized after drug treatment. SJW in present study normalizes brain 5-HT by decreasing tryptophan availability to the brain. A decrease in 5-HIAA levels predicts increased release as SJW at low doses, act as MAO inhibitor has not been established (Muller *et al*., 1997) It is therefore concluded that the precursor dependency of 5-HT and its behavioral consequences probably have a survival value.

Future studies on the effects of drug on circulating corticosterone concentrations and Tryptophan/LNAA ratio will help to understand its mechanism of action in controlling brain tryptophan levels.

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