Chronic administration of St. John's Wort attenuates alcohol intake and brain indoleamine 2, 3-dioxygenase activity in mice

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Abstract: Present study aims to elucidate the effects of chronic administration of St. John's Wort (SJW) (500mg/kg) on brain tryptophan (TRP) metabolites and indoleamine 2-3 dioxygenase (IDO) activity in alcohol treated mice. Locally bred Albino BALB/c mice, weighing 20-25g were divided into three groups (untreated controls, Alcohol, Alcohol +Drug) having 6 mice in each. Freshly prepared ethanol solution was administered in drinking water in the proportion of 5% for three days or 8% for 3 weeks to two groups. After 3 weeks drug group was treated with SJW (dissolved in ethanol: saline 1:3 v/v) at a dose of 500mg/kg was administered orally for 1 week. During treatment alcohol intake was monitored .In present finding chronic administration of SJW significantly reduced ethanol intake by 78.6% ($P<0.001$) in mice. Data analyzed by student's t-test indicates that SJW remarkably reduce kynurenine (KYN) by 60.9% ($P<0.001$) and KYN/TRP ratio (IDO) activity) by 70.9% ($P<0.001$) in brain. Low serotonin level promotes alcohol intake. Present results suggest that SJW decreases alcohol intake by inhibiting IDO thereby shifting TRP catabolism towards serotonin synthesis.

Keywords: Alcohol, SJW, tryptophan, kynurenine, IDO.

INTRODUCTION

Indoleamine 2-3 dioxygenase (IDO) is a glycoprotein having heme in its structure; it has two isoforms IDO- 1 and IDO-2 (Fujiwara *et al.,* 1978). It is found in multiple tissues and cells such as intestine, stomach, lungs macrophages, monocyte, (Taylor and Feng, 1991) and brain (Guillemin *et al.,* 2007). IDO mainly catabolise tryptophan (TRP) via Kynurenine (KYN) pathway but it acts on other indoleamines as well such as tryptamine, serotonin and melatonin (Ball *et al.,* 2007). It is induced by cytokines and inflammatory molecules (Guillemin *et al.,* 2003), but it is mainly regulated by interferon gamma (Taylor and Feng, 1991). The serum KYN/TRP ratio indicates IDO activity; it is induce by inflammatory cytokines (Schroksnadel *et al.,* 2006; Raison *et al.,* 2010). Alcohol abuse and alcoholism caused systemic and central nervous system (CNS) inflammation that relates with immune system (Irwin and Miller, 2007). Misuse of alcohol for prolong period cause rise in cytokines of circulation as well as CNS (Crews *et al.,* 2006; Crews and Nixon, 2009). Neuroinflammation as a result of alcohol abuse stimulates IDO and subsequently cause depression (Kelley and Dantzer, 2011). The activation of IDO shifts the catabolism of TRP from serotonin (5HT) to kynurenine (KYN) formation that results in low level of 5HT and leads to depression. Saint John's wort (SJW) (*Hypericum perforatum*) is known for its beneficial effects to treat mild to moderate depression is an herbal medicinal plant. It has been used as a folk medicine in many European countries. Hypericin of Saint John's Wort is documented for its antidepressant activity in several clinical and preclinical studies (Werenke, 2004). Along with its antidepressant property, Hyperforin also possess powerful anti-inflammatory activity (Medina *et al.,* 2006). *In vitro* hyperforin inhibits many proinflammatory functions of leukocytes like chemotaxis and chemoinvasion because of its anti-inflammatory activity (Dell' Aica *et al.*, 2007; Lorusso *et al.*, 2009). Extracts of SJW are known to have important features to reduce alcohol-induced depression. Preclinical studies on animal models reported that pretreatment with Hypericum perforatum extract (HPE) reduced alcohol fondness in alcohol preferring strains of mice and rats (Rezvani *et al.*, 1999; Perfumi *et al.,* 1999; Wright *et al.*, 2003). Many studies reported that low serotonin level in brain promotes high alcohol consumption (Murphy *et al.*, 1982; Higley *et al.*, 1996). Studies on animal reported that drugs that elevate post-synaptic concentration of 5-HT either by inhibiting 5-HT reuptake (Naranjo *et al.,* 1990; Le Marquand *et al.*, 1994; Maurel *et al.,* 1999), or by increasing its secretion (Higgins *et al.,* 1992) reduce ethanol consumption. HPE increases the concentration of 5HT, γ-amino butyric acid, nonepinephrine, and dopamine in the central nervous system and its antidepressant properties related to its serotonergic or dopaminergic properties (Butterweck *et al.*, 1997; Muller *et al.,* 1997).

MATERIALS AND METHODS

Chemicals and reagents

Tryptophan, kynurenine and quinolinic acid (QA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Saint John's Wort was obtained from Medics Pharma, Pakistan. Ethanol and methanol (HPLC grade). All others chemicals used were of highest analytical grade.

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Animals and treatment

All animals were treated according to the rules of national research council for the care and use of laboratory animals (1996). From institutional animal ethics committee, University of Karachi, ethical approval was obtained. Locally bred Albino BALB/c mice, weighing 20-25g at the start of experiment were kept 6 per cage under natural 12h dark/light cycle at 22±3^{o}C. The animal had given solid lab chow and water *ad libitum* to accommodate with their environment before starting the experiment. Mice were divided into three groups (untreated controls, Alcohol, Alcohol +Drug) 6 in each. Daily freshly prepared ethanol solution was administered in drinking water in the proportion of 5% for three days or 8% for 3 weeks to two groups. After 3 weeks drug group was treated with SJW (dissolved in ethanol: saline 1:3 v/v) at a dose of 500mg/kg was administered orally for 1 week. Drug was freshly prepared each day. During treatment alcohol intake of Alcohol and Alcohol +SJW groups was monitored. Mice were killed and brains were immediately isolated and frozen in liquid nitrogen and were stored at -70^{o}C until analysis.

Neurochemical estimations

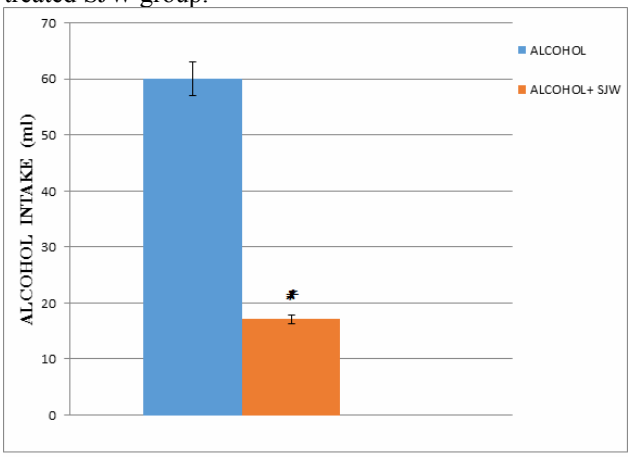
TRP and its metabolites in brain was analyzed by using HPLC-UV/FL. Whole brain was weighed (0.5g) and taken in homogenizing tube, 0.5ml of ice-cold HPLC-grade H_2O and 1ml of 12% $HClO_4$ were added then mixture was homogenized for approximately 5 seconds. Homogenates were incubated in ice cooled rack for 10 minutes. Centrifuge for 10 minutes at 6000 rpm at 4^{o}C. Then supernatant was taken into another tube and volume was made up to 2ml with 6% $HClO_4$. 1ml of extract was stored at -70^{o}C for analysis. In present work isocratic HPLC was used for the separation and estimation of TRP and its metabolites (quinolinic acid and kynurenine). The system comprised of different components such as ultraviolet and fluorescence detector (RF 20A, Shimadzu), pump (LC 20 AT), an injector (20µl loop) connected to the LC- computerized program and stationary phase C18 reverse phase column was used (25mm × 0.26mm internal diameter, 5µm average particle size, Tecknochroma). For TRP and KYN separation, a mobile phase of 27% methanol: 73% 10mM sodium dihydrogen phosphate final pH of 2.8 was used at a flow rate of 1.0-1.2ml/min. Wavelength of ultraviolet (UV) detector was set on 220nm and excitation and emission of fluorimetric detector was 254nm and 404nm respectively. For separation of quinolinic acid same mobile phase of final pH 2.0 was used at a flow rate of 1.15ml/min only ultraviolet detector (220nm) was used (Badawy and Morgan 2010).

STATISTICAL ANALYSIS

Results are presented as mean ± SEM. Two tailed student's t-test was used to analyzed data. All results were considered significant if the associated P (probability of error) values less than 0.05

RESULTS

Fig. 1 shows the effect of chronic administration of SJW on alcohol consumption. Data analyzed by student's t-test shows significant decreased (78.6%, P<0.001) in alcohol intake when alcohol group was compared with alcohol treated SJW group.



Experimental details are given in material and methods section. All values are mean ± SEM of six mice in each group. Statistical analysis was performed using two-tailed student's t-test. *P<0.001indicate significant difference when alcohol compared with alcohol + SJW mice.

Fig. 1: Effect of sjw on alcohol intake

Table 1 shows the effect of alcohol consumption on brain TRP metabolites in mice. It was found that brain tryptophan was significantly decreased (36%, p<0.05) while Kynurenine and KYN/TRP ratio (IDO activity) was significantly increased by 60% (p<0.01and by 148% (p<0.05) respectively with no effect on brain QA. table 1 also shows effect of SJW on brain TRP metabolites in alcohol treated mice. There was significant decrease in brain KYN (60.9%, p<0.001) and KYN/TRP ratio (70.9%, p<0.001) but there were no effect on brain TRP and QA.

DISCUSSION

Present study shows decreases in brain tryptophan after 4 weeks alcohol consumption. Previous studies have demonstrated that a decrease in tryptophan usually occurs with alcohol consumption. This decrease occurs with or without a meal and is identified by observing the ratio of tryptophan to other amino acids it competes with for uptake into the brain. One study of alcohol's effect on tryptophan metabolism suggests that TDO may be involved in the loss of tryptophan after alcohol consumption (Badawy *et al*., 2009). In present finding chronic administration of SJW (500mg/kg) remarkably decreases ethanol consumption in mice. Our result is

Table 1: Effects of SJW on brain tryptophan metabolites in alcohol treated mice

Parameters	Untreated Controls	Alcohol	Alcohol + SJW
Tryptophan (µg/g)	3.2± 0.18	2.04 ± 0.36*	2.5 ± 0.22
Kynurenine (µg/g)	0.83± 0.02	1.33±0.08**	0.52±0.09†
Quinolinic Acid (nmole/g)	1.01± 0.10	1.30 ± 0.24	1.19 ± 0.18 NS
KYN/TRP Ratio	0.25± 0.08	0.62±0.09*	0.18±0.02†

Experimental details are given in material and methods section. All values are mean ± SEM of six mice, in each group. Statistical analysis was performed using two-tailed student's t-test. N.S. indicates the non-significant difference. Significance difference is indicated by *p<0.05, ** p<0.01when alcohol treated group was compared with untreated controls and †p<0.05 when Alcohol + SJW group of mice was compared with Alcohol treated group.

similar to De Vry *et al.,* 1999; Perfumi *et al.*, 1999, 2001; Rezvani *et al.,* 1999; Panocka *et al.*, 2000 that *hypericium perforatum* extract (HPE) inhibits ethanol intake in alcohol-preferring rats. Alcoholism and depression share the same neurochemical mechanisms (Markou *et al.,* 1998). Many studies reported that low serotonin level in brain promotes high alcohol consumption (Murphy *et al.*, 1982; Higley *et al.,* 1996). Animal studies stated that drugs that raise post-synaptic serotonin levels either by inhibiting its re-uptake (Borg *et al.*, 1985; Naranjo *et al.,* 1990; Le Marquand *et al.,* 1994; Maurel *et al.*, 1999) or by raising its release (Higgins *et al.,* 1992) reduce ethanol consumption. HPE increases the concentration of 5HT, nonepinephrine, and dopamine and γ-amino butyric acid in the brain and its antidepressant properties related with its dopaminergic or serotonergic activity (Butterweck *et al.,* 1997; Muller *et al.,* 1997).

In present study chronic administration of SJW (500mg/kg) in alcohol treated mice remarkably reduce KYN concentration and KYN/TRP ratio in brain. KYN/TRP ratio represents the IDO activity. Serotonin and KYN metabolites synthesized from TRP. The activation of IDO depresses the synthesis of 5-HT through the utilization of TRP which can contribute to the development of depression. In contrast, the inhibition of IDO activation inhibits the appearance of depressive- like behavior. Moreover, KYN administration to wild type mice induced depressive -like behavior dose dependently (O'Connor *et al*., 2009). Acute oral administration of SJW at the dose of 250-500 mg/kg increases serotonin, norepinephrine and dopamine in the brain (Calapai *et al.,* 2001). Long term treatment by SJW (500mg/kg) increased the 5-HT in hypothalamus, hippocampus and amygdala in swim stressed and unstressed rats (Butterweck *et al.,* 2002; Bano *et al.,* 2014).

CONCLUSION

Present results suggest that SJW by inhibiting IDO shift TRP catabolism towards serotonin synthesis may help to reduce depressive behavior in alcohol dependent mice.

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