INTRODUCTION

Extracts of Hypericum perforatum L. (St John’s Wort, SJW) have been used since antiquity for the treatment of mild to moderate depression and have recently been studied clinically (Ernst, 1995; Melchert, 1996; Nordfors and Hartvig, 1997; Volz, 1997). A meta-analysis of randomized clinical trials has shown that SJW is superior to placebo, equally as effective as standard antidepressants, and has a clear advantage over the latter in terms of side-effects for the treatment of mild to moderate depression (Ernst, 1995; Melchert, 1996). It has been shown that SJW extract elevates the level of serotonin, dopamine (DA), noradrenaline (NA), and γ-aminobutyric acid (GABA) in the brain and the antidepressant effects of SJW have been suggested to be associated with its serotonergic and/or dopaminergic properties (Butterweck et al., 1997; Muller et al., 1997).

Alcoholism and depression have some common neurochemical substrates (Markou et al., 1998). It has been speculated that the pathophysiology of alcoholism and depression might involve pre-existing low brain serotonin levels that are increased transiently by alcohol consumption (Ballenger et al., 1979; Rosenthal et al., 1980). Serotonergic compounds have been shown to reduce pathologic drinking in experimental animals (Murphy et al., 1988; Rezvani et al., 1991; Sellers et al., 1992; Overstreet et al., 1994; Rezvani and Grady, 1994; Tomkins et al., 1995) and heavy drinkers (Lawrin et al., 1986; Naranjo et al., 1990) and alleviate the symptoms of depressive disorders (Meltzer, 1990). Because of this similarity in the pathogenesis of depression and alcoholism and the antidepressant properties of SJW, we postulated that the SJW may also reduce voluntary alcohol intake in alcohol-prefering rats. To test this hypothesis, we studied the effects of the SJW extract on voluntary alcohol intake in two different strains of alcohol-prefering rats: inbred alcohol-prefering fawn-hooded (FH) rats and selectively-bred high-alcohol drinking (HAD) rats.
MATERIALS AND METHODS

Animals

Two different genetic animal models of human alcoholism were used: the selectively-bred HAD rats developed (Li and McBride, 1995) at, and obtained from, Indiana University and the alcohol-prefering inbred FH rats developed and maintained at the University of North Carolina School of Medicine at Chapel Hill (Rezvani et al., 1990). The original stock of FH rats was obtained from the New York Department of Health in 1989 (courtesy of Dr Jean Dodd). Both strains have been widely used to study the effects of different compounds on voluntary alcohol intake (Murphy et al., 1988; Rezvani et al., 1991, 1997; Rezvani and Grady, 1994; Overstreet et al., 1997).

Adult male rats were housed individually in wire mesh cages (26 × 34 × 20 cm) under a constant room temperature of 22 ± 1°C and a 12:12 light–dark cycle (10:00–22:00, dark). Animals were fed Agway Prolab Rat/Mouse/Hamster 3000 formula (Agway, Syracuse, NY) and water ad libitum.

Establishment of baseline alcohol intake

Alcohol intake was determined using the standard two-bottle choice method utilized in our and other laboratories for many years (Murphy et al., 1988; McBride et al., 1990; Rezvani et al., 1990, 1991, 1997). Animals were first given free access to water in a graduated Richter tube for 1 day. Next, they were given access only to a solution of 10% (v/v) ethanol for 3 consecutive days. During this period, rats became accustomed to drinking from Richter tubes and to the taste and pharmacological effects of alcohol (Waller et al., 1982; Rezvani et al., 1997). Thereafter, they were given free access to both water and a solution of 10% alcohol for at least 3 consecutive weeks and throughout the period of study. Treatments were administered orally in the morning (09:30). Water and alcohol intake were recorded at 6 and 24 h after the treatment, whereas food intake was measured at 24 h. Water and alcohol intakes were measured by reading the numbers on the graduated Richter tubes and subtracting them from the previous readings.

Preparation of agents

Solutions of SJW extract were prepared in distilled water. The volume of vehicle (distilled water) or SJW extract administered orally was 5 ml/kg body wt. The standardized compound (LI 160, batch no. 970201 provided by Lecthwere Pharma AG, Berlin) is an 80% methanolic extract of Hyperici herba and contained 0.22% total hypericin (sum of hypericin and pseudohypericin) measured by thin-layer chromatography and 4.05% hyperforin measured by high-performance liquid chromatography. These data were provided by Lecthwere Pharma AG, Berlin. Solutions of 10% alcohol were prepared from 95% grade ethanol and distilled water.

Experimental protocol

Acute administration. After establishment of a stable baseline for alcohol, food, and water intake, FH (n = 8–10) and HAD (n = 8) rats were administered by gavage at 09:30 either the vehicle or one of the following doses of SJW extract: 100, 200, 400, and 800 mg/kg (for FH rats) and 100, 200, 400, and 600 mg/kg (for HAD rats) using a random treatment order. Each rat received the vehicle and all of the doses of SJW extract. The interval between treatments was at least 3 days. The solutions of extract were delivered slowly into the stomach through a special ball-tipped 16 gauge stainless steel gavage needle (Beckton Dickenson) (Rezvani et al., 1986).

Chronic administration. In another experiment, to determine the effect of chronic administration of SJW on alcohol intake, a group of FH rats (n = 9) was given orally once a day either the vehicle or 400 mg/kg of SJW extract for 15 consecutive days.

Statistical analysis of data

The results are presented as means ± SEM. Alcohol intake (g/kg) was calculated by multiplying the volume of alcohol consumed in ml by 10% and 0.7893 (ethanol density)/body wt in kg. Alcohol preference, expressed as a percentage, was calculated as follows (volume of alcohol consumed in ml/total fluid intake in ml) × 100 (Rezvani and Grady, 1994; Rezvani et al., 1997). Statistical differences between drug-treated and control groups were determined by using ANOVA and Tukey Student’s t-test for multiple comparison (GB-STAT, version 5.01, Dynamic Microsystems, Inc., Silver Spring, MD, USA).

RESULTS

Acute administration

Compared with the control vehicle, a single oral administration of different doses of SJW extract
significantly attenuated alcohol intake in both HAD (Fig. 1A) and FH rats (Fig. 1B) at the 6-h time point. In data not shown, we found that there was less suppression at 24 h.

In addition to their maximal effects at 6 h (Fig. 1), the higher doses of SJW (800 mg/kg for FH rats and 400 and 600 mg/kg for HAD rats) suppressed alcohol intake to the greatest extent also at 24 h (Fig. 2A and B). Overall, there was a trend for water intake to be increased in both FH and HAD rats. However, this trend did not reach statistical significance. The combination of a significant reduction in alcohol intake and a moderate increase in water intake, however, led to a significant ($P < 0.01$) reduction in alcohol preference only at 6 h with highest doses of SJW in both FH and HAD rats. Alcohol preference decreased from a 3-day pre-treatment baseline of 78 to 48% in FH rats and from a baseline of 90 to 18% for HAD rats. However, alcohol preference did not significantly change with low or medium doses of SJW in either strain.

Acute administration of the SJW extract did not significantly influence food intake in FH rats (Fig. 3B), but at two high doses reduced food intake

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**Fig. 1.** Effects of acute oral administration of vehicle and different doses of St John’s wort (SJW) extract on the 6-h intake of 10% (v/v) alcohol in high-alcohol drinking (HAD) and fawn-hooded (FH) rats.

Data are means ± SEM [n = 8 for HAD (A) and 8–10 for FH (B) rats]. $F(4,36) = 9.0$, $P < 0.0001$ for FH rats and $F(4, 28) = 18$, $P < 0.0001$ for HAD rats. **$P < 0.05$, ***$P < 0.01$ vs vehicle.

**Fig. 2.** Effects of acute oral administration of vehicle and different doses of St John’s wort (SJW) extract on the daily intake of 10% (v/v) alcohol in high-alcohol drinking (HAD) and fawn-hooded (FH) rats.

Data are means ± SEM [n = 8 for HAD (A) and 8–10 for FH (B) rats]. $F(4,28) = 7$, $P < 0.0005$ for HAD rats and $F(4,36) = 2.7$, $P < 0.04$ for FH rats. **$P < 0.05$, ***$P < 0.02$, vs vehicle.
in HAD rats (Fig. 3A). Although this reduction in food intake was statistically significant, it was significantly ($P < 0.001$) of a much lesser extent, compared with that for alcohol intake (71 and 93% reduction in alcohol intake from 3-day baseline vs 20 and 27% in food intake from 3-day pretreatment baseline for the two high doses of SJW extract used).

*Chronic administration.* It has been shown that tolerance develops to the suppressing effects of other drugs on alcohol intake (Rezvani et al., 1992). To test this phenomenon with SJW extract, a chronic experiment was conducted with nine adult male FH rats. After establishment of stable baseline for alcohol ($6.7 \pm 0.45 \text{ g/kg/day}$) and water intake, an intermediate dose of 400 mg/kg of SJW extract or vehicle was given to FH rats orally once a day for 15 consecutive days. As can be seen from Fig. 4, repeated oral administration of SJW extract, but not vehicle, for 15 consecutive days in FH rats significantly reduced the 24-h alcohol intake.

Alcohol preference at 6 h, but not at 24 h, was also reduced significantly after chronic administration of SJW extract (Fig. 5). Chronic administration of SJW did not significantly change either water or food intake at 24 h in FH rats (data not shown). However, although chronic administration of SJW extract did not significantly change food intake, it reduced total caloric intake at 24 h. Reduction in caloric intake reflects a significant reduction in daily alcohol intake, which contains 7.2 kcal/g.

### DISCUSSION

The present study shows that an acute oral administration of SJW extract can significantly and dose-dependently reduce voluntary alcohol intake in two different strains of alcohol-prefering rats. Further, tolerance to this effect of SJW did not develop after chronic oral administration of this extract in FH rats. The mechanism of action of the extract on alcohol intake cannot be explained with the present data. *Hypericum* extract contains at least...
10 constituents or groups of components that may contribute to its pharmacological effects (Bladt and Wagner, 1994; Cott, 1997; Nahrstedt and Butterweck, 1997). There is some evidence that two active ingredients in the extract, hypericin and hyperforin, block reuptake of serotonin, DA, NA, and GABA (Muller et al., 1997; Chatterjee et al., 1998). Low brain levels of both serotonin and DA have been implicated in alcohol-seeking behaviors (DiChiara and Imperato, 1985; McBride et al., 1990; Sellers et al., 1992; Samson et al., 1993; Weiss et al., 1993; Rezvani and Grady, 1994). An innate deficiency of serotonin and/or DA leads to excessive alcohol drinking in experimental animals (Rezvani et al., 1990; LeMarquand et al., 1994). It has also been shown that GABAergic mechanisms, particularly those associated with GABA_\text{A} receptors, may be involved in the acquisition of voluntary alcohol intake in laboratory rats (Hyttia and Koob, 1995). Thus, it is possible that the SJW extract exerts its attenuating effects on voluntary alcohol intake by increasing serotonin and DA levels in the synaptic cleft by blocking the reuptake of these transmitters. In agreement with this hypothesis, Muller et al. (1997) showed that Hypericum extract is a rather potent inhibitor of synaptosomal serotonin reuptake, and, in contrast to most other antidepressant drugs, the extract also inhibits the synaptosomal uptake of DA and NA with similar potencies. It has also been demonstrated that hyperforin, the major lipophylic chemical constituent of SJW, inhibits GABA uptake with an IC_{50} value of about 0.5–0.10 mg/ml in synaptosomal preparations (Chatterjee et al., 1998). The fact that the extract of SJW shows a similar affinity for DA, serotonin, NA, and GABA transporter systems might be the basis for its broad psychopharmacological effects.

Suzuki et al. (1984) reported that hypericin inhibited both monoamine oxidase-(MAO) A and B; however, this finding could not be confirmed by other workers (Demisch et al., 1989; Bladt and Wagner, 1994). The latter group demonstrated that none of the fractions of Hypericum or their constituents tested in vitro showed significant MAO inhibition at pharmacologically relevant concentrations. Although some of the discrepancies could be the result of using different extracts that might be different in purity, existing data suggest that the pharmacological actions of SJW extract cannot be explained by MAO inhibition.

One may consider also whether SJW exerts its action on alcohol intake by interfering with alcohol metabolism. Although the present data do not address this possibility, other investigators have found that intragastric administration of the extract of SJW does not interfere with alcohol metabolism (Perfumi et al., 1999, personal communication).

It has been shown that the number of 5-HT_{1A} and 5-HT_{2A} receptors are significantly increased by 50% in Hypericum-treated rats (Teufel-Mayer and Gleitz, 1997). Interestingly, both 5-HT_{2} and 5-HT_{1A} receptors have been implicated in excessive drinking in rats (McBride et al., 1993; Overstreet et al., 1994, 1997). Thus, it is possible that SJW extract reduces voluntary alcohol intake by acting directly at these receptors and therefore altering 5-HT transmission.

The fact that SJW extract increases 5-HT_{2} receptors during chronic treatment (Teufel-Mayer and Gleitz, 1997) indicates that SJW may also be a novel antidepressant agent. Typically, antidepressant agents lead to the downregulation of 5-HT_{2} receptors (Maies and Meltzer, 1995). However, chronic treatment with SJW extract leads to an increase in 5-HT_{2} receptors (Teufel-Mayer and Gleitz, 1997).
as well as an antidepressant effect (Ernst, 1995). Indeed, we found that FH rats, which exhibit an innate high immobility in the forced swim test of depression (Rezvani et al., 1990), became less immobile following chronic (15 consecutive days) treatment with SJW extract (Rezvani et al., 1998). Others have also recently reported suppression of alcohol intake after acute administration of SJW extract and reduction in swim test immobility after chronic treatment (Perfumi et al., 1999). Perhaps the downregulation of 5-HT2 receptors induced by chronic classic antidepressant treatment accounts for their relative lack of efficacy in the treatment of alcoholism (Litten et al., 1996). Indeed, in contrast to the constant suppression of alcohol intake seen during chronic treatment with SJW extract, we have observed that tolerance develops to the suppressive effects of the serotonin reuptake inhibitor, fluoxetine, on alcohol intake (Rezvani et al., 1992). It appears, therefore, that 5-HT2 receptors may not be involved in the alcohol-suppressant effects of SJW, but changes in these receptors may account for the lack of tolerance development following chronic treatment.

In conclusion, the present findings demonstrate that acute or repeated oral administration of the SJW extract induced a dose-dependent reduction in alcohol intake in alcohol-prefering rats. Although the antidepressant effect of SJW extract has been suggested to be associated with its serotonergic property, its effect on alcohol intake is probably linked to its broad spectrum of pharmacological properties, such as reducing the re-uptake of serotonin, DA, NA, and GABA transmitters in the brain. The exact mechanism of action of the extract on alcohol intake remains to be investigated systematically.

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REFERENCES


