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# Behavioral profile of *Hypericum perforatum* (St. John's Wort) extract. A comparison with standard antidepressants in animal models of depression

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**Abstract. – BACKGROUND AND OBJECTIVES: *Hypericum (H.) perforatum*, popularly called St. John's Wort has been used traditionally for the treatment of anxiety, depression and as a nerve tonic. Large amount of clinical and animal experimental data demonstrate that *H. perforatum* acts by biochemical mechanisms similar to the tricyclic antidepressants or serotonin reuptake inhibitors. However, its efficacy in comparison to standard antidepressant drugs is not well studied. The present study evaluated *H. perfortum* extract in animal models of depression compared to clinically used antidepressants.**

**MATERIALS AND METHODS: The effects of standardized extract of *H. perforatum* was compared with standard antidepressants using animal models of depression such as forced swim test (FST), yohimbine induced lethality test, pnetylenetetrazole (PTZ) induced convulsion and locomotor activity tests. Different doses of the plant extract and standard drugs were administered to rats or mice intraperitoneally (i.p).**

**RESULTS: In the FST, *H. perforatum* extract (30-90 mg/kg i.p.) caused a dose dependent reduction in immobility time in rats with maximal effect being 53% at 90 mg/kg. This effect was reversed at higher doses (100 mg/kg) showing a U-shaped dose response curve. Fluoxetine and imipramine (30-70 mg/kg i.p.) produced similar reduction in the immobility time in rats. Venlafaxine exhibited weak antidepressant effect. *H. perforatum* extract (30-100 mg/kg i.p.), dothiepin (10-50 mg/kg i.p.), fluoxetine (30-60 mg/kg i.p.) and venlafaxine (20-40 mg/kg i.p.) potentiated yohimbine induced lethality. PTZ induced toxicity was also enhanced with these agents. In the locomotor activity test *H. perforatum* decreased the locomotor counts of mice similar to standard antidepressants.**

**CONCLUSIONS: *H. perforatum* has antidepressant properties similar to standard antidepressants. The antidepressant profile of *H. perforatum* is closely related to the selective serotonin reuptake inhibitors class of antidepressants.**

*Key Words:*
*Hypericum perforatum*, St. John's Wort, Anti-depressants, Forced swim test..

## Introduction

*Hypericum (H.) perforatum*, popularly called St. John's Wort in traditional medicine system, it is well known medicinal plant which has been used for centuries for a range of indications including skin wounds, eczema, burns and psychological disorders[1,2]. The plant has been used traditionally for the treatment of excitability, neuralgia, anxiety, depression and as nerve tonic[3]. Currently antidepressants effects of *H. perforatum* extract have been demonstrated by several animal experiments and clinical studies[4-6]. The data from these studies show that *H. perforatum* extract have inhibitory effect on immobility in rats forced swim test (FST) and is clinically effective as antidepressant medicine for mild to moderate type of depression[7]. Growing number of investigations demonstrate that *H. perforatum* acts by biochemical mechanisms similar to the tricyclic antidepressants or serotonin reuptake inhibitors[8,9]. It has been found to be safe and devoid of serious side effects particularly compared to tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs)[10,11].

The phytochemical studies have revealed number of biologically active compounds including hypericin, hyperforin, hyperoside and pseudohypericine[12,13]. Hypericin and hyperforin are considered the main principles responsible for the antidepressant activity of St. John's Wort[9,14].



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*Hypericum perforatum* has long been recognized as a treatment for mild to moderate depression; however, the data concerning its comparative animal studies with standard antidepressants are scarce. Moreover, the constituents of Hypericum extract differ between the individual manufacturers and the phytochemical diversity influences the efficacy of Hypericum preparation. Keeping this in mind the current study was planned to evaluate the recently marketed Hypericum preparation in Pakistan for its antidepressant efficacy in animal models of depression. We have previously reported that this Hypericum preparation exhibited better efficacy than placebo in a double blind clinical study[15]. In the current investigation the antidepressant effect of standardized Hypericum preparation was assessed in comparison to standard anti-depressant drugs in animal models of depression. There are variety of behavioral animal models of depression including FST, locomotor activity, yohimbine and pentylenetetrazole (PTZ) induced lethality tests, that are used to screen and assess the anti-depressant effects of natural agents and synthetic compounds[16]. These behavioral paradigms were employed in the our current investigation to know the efficacy and mechanism of action of *H. perforatum* in comparison to clinically used anti-depressant drugs.

## Materials and Methods

### Animals

Male NMRI mice (20-30 gm) and Sprague-Dawley/ Wistar rats (200-300 gm) were obtained from animal house facility of HEJ Research Institute of Chemistry University of Karachi, Pakistan. The animals were housed ten per cage under standard condition with 12h light: dark cycle with free access to food and water.

### Chemicals

Fluoxetine (Sigma USA), Venlafaxine (Wyeth-Lederle Karachi, Pakistan), Dothiepin HCl (Knoll Pharma. Karachi, Pakistan), Diazepam (Roche Pharma, Karachi, Pakistan), Pentylenetetrazole, Imipramine, Yohimbine and all the other chemicals used were of analytical grade and were purchased from Sigma-Aldrich St. Louis, MO, USA.

### Hypericum Perforatum Extract

The plant extract of *Hypericum perforatum* (standardized on 0.3% Hypericin contents) was a gift from Medics Laboratories, Karachi, Pakistan.

### Drug Preparation and Administration

All standard antidepressants were dissolved in saline (0.9% NaCl) except *H. perforatum* that was solubalized in 10% dimethylsulfoxide (DMSO). Treatment were given i.p., as clear solution or suspension in a volume 10 ml/kg and 1 ml/kg for mice and rats respectively with exception of yohimbine that was administered subcutaneously (s.c.) in volume of 5 ml/kg.

## Experimental Methods

### Acute Toxicity Test

Male mice (25-30 g) were injected i.p. with vehicle (saline or 10% DMSO) or different doses of *H. perforatum* extract or standard anti-depressants for three consecutive days. Animals were observed for 1-3 hours for any changes in their behavioral activity and the neurotoxic effects. Mortality occurred within one week after treatment was recorded and $LD_{50}$ value (Lethal dose causing 50% mortality in experimental animals) was calculated wherever applicable.

### Forced Swim Test (FST)

This test was performed using the method described previously[17]. Male rats (200-260 g) were placed individually into container (height 45cm, diameter 17 cm) containing 20 cm water at 25-30°C. Animals were left to swim in the water for 15 minutes followed by gentle drying and returned to their cages. After pre-session animals received either vehicle or test samples 1 hr prior to second swimming exposure 24 h later. In the second exposure rats were allowed to swim for duration of 5 minutes and immobility time was recorded. Immobility was considered as complete absence of active escape oriented behaviors such as swimming, diving, rearing and sniffing.

### Yohimbine Induced Lethality Test

Male mice weighing 25-30 g were treated i.p. with either vehicle or different doses of test compound 1hr prior to administration of yohimbine (20 mg/ kg s.c.). Mice were observed for 1-2 hrs for behavioral changes and acute symptoms of toxicity. Mortality occurred during 18 hrs post yohimbine was registered[16,18].

### Pentylenetetrazole (PTZ) Induced Convulsions

Male mice (25-30 gm) were injected saline or test compounds. Sixty minutes later animals

were dosed with 60 mg/ kg i.p. pentylenetetrazole[19-20] and observed for 30 min. The latency of convulsions and the number of deaths occurred with in 30 minutes after pentylenetetrazole administration were recorded.

### Locomotor Activity Test

This test was carried out according to the method as described elsewhere[21], using Optovarimex Minor (Columbus Instruments, Columbus, OH, USA). The instrument consists of transparent plastic box having horizental and vertical sensors with infrared emitters, placed perpendicular to each other. The horizontal and vertical movements of the animals causing interruption of infrared beam are displayed as numerical counts.

Mice of either sex were administered vehicle or test compounds 1 hour before placement in activity cage. After acclimatization time of 15 min six observations (10 min each) of locomotors count were recorded[16]. Experiments were conducted between 9:00 and 10:00 a.m. to avoid any diurnal effect on motility of the animal.

### Statistical Analysis

The data are expressed as means ± SEM. Statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple comparison test when significant differences were present. $p < 0.05$ was considered statistically significant.

## Results

### Acute Toxicity

Hypericum extract caused about 58% mortality at the dose of 500 mg/kg and at 1000 mg/kg 100% mortality was observed, $LD_{50}$ was 450 mg/kg. The $LD_{50}$ values for fluoxetine, dothiepin and venlafaxine was 74, 95 and 82 mg/kg i.p. respectively (Table I).

### Forced Swim Test (FST)

*Hypericum perforatum* extract induced a dose dependent reduction in the immobility time in rats between 30-90 mg/kg i.p. with the maximal effect being 53% at 90 mg/kg. However, above this dose (100 mg/kg) its effect was reversed, showing U-shaped dose response relationship (Figure 1).

As shown in Figure 2, the standard antidepressant drugs; fluoxetine, imipramine and venlafaxine were also tested at the dose of 30, 50 and 70 mg/kg i.p. Among these drugs imipramine was

**Table I.** Acute toxicity test of H. perforatum and standard anti-depressant drugs in mice.

| Treatment | Dose (mg/kg) i.p. | Percent mortality | $LD_{50}$ (mg/kg) |
|---|---|---|---|
| *H. perforatum* | 100 | 0 | 450 |
| | 200 | 0 | |
| | 500 | 58 | |
| | 800 | 83 | |
| | 1000 | 100 | |
| Fluoxetine | 30 | 0 | 74 |
| | 60 | 0 | |
| | 70 | 43 | |
| | 80 | 71 | |
| | 90 | 100 | |
| Dothiepin | 30 | 0 | 95 |
| | 60 | 0 | |
| | 80 | 20 | |
| | 90 | 33 | |
| | 100 | 67 | |
| | 120 | 100 | |
| Venlafaxine | 30 | 0 | 82 |
| | 60 | 0 | |
| | 70 | 33 | |
| | 80 | 43 | |
| | 90 | 83 | |
| | 100 | 100 | |
| Control | Saline (10 ml/kg) | 0 | – |

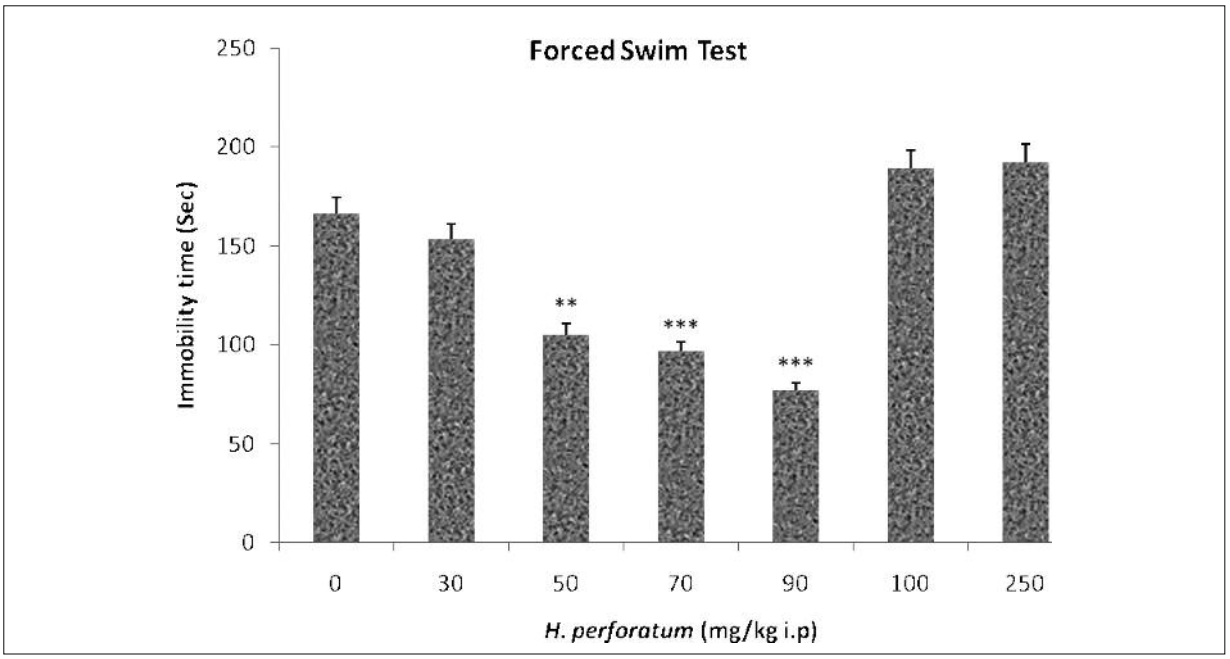


**Figure 1.** Effect of *H. perforatum* on immobility time in rat forced swim test. Rats were administered (i.p.) different doses of the plant extract as a single i.p. injection, 1 hour prior to the test. Values represent immobility time in seconds. Data represent mean ± SEM (n= 6-10). Asterisks indicate level of significance as $^{**}p < 0.01$ and $^{***}p < 0.001$, One way ANOVA followed by the Student-Newman-Keuls multiple comparison test.

the most potent producing about 55% reduction in immobility time in rats at 30 mg/kg i.p. Further increase in the dose caused a decrease in efficacy of imipramine in the FST. Fluoxetine exhibited dose dependent effect (30-70 mg/kg i.p.) reducing the immobility time of rats from 166.4 ± 5 (control group) to 82.3 ± 10 (fluoxetine treated group) with maximum reduction of 50% at 70 mg/kg i.p. Venlafaxine brought about 34% reduction in the immobility time of rats and its effect was decreased with the increase in dose (Figure 2).

### Yohimbine Induced Lethality Test

Preliminary experiments were conducted to select the dose of yohimbine for the evaluation of test compounds and to determine the toxicity of yohimbine under our experimental conditions. Yohimbine caused a dose dependent increase in mortality of mice. At 20 mg/kg s.c. it produced 7% mortality of mice and this dose was selected for further experiments.

Hypericum extract had no effect at 10 mg/kg i.p. but potentiated yohimbine induced toxicity in dose dependent manner showing 33% mortality at the dose of 30 mg/kg i.p. While at 60 and 100 mg/kg. the mortality observed was 69 and 100% respectively (Table II).

Fluoxetine caused 100% mortality at 60 mg/kg i.p. Dothiepin exhibited dose dependent potentiation of yohimbine toxicity with 17% mortality at 10 mg/kg reaching to 71% at 50 mg/kg i.p. Venlafaxine was found to be the most potent compound producing 100% mortality of mice at 40 mg /kg i.p. (Table II).

### Pentylenetetrazole (PTZ)-Induced Convulsion Test

Table II demonstrates that *H. perfpratum* extract significantly reduced the latency of convulsion and increased mortality of mice. It caused maximum reduction in latency of about 43% at the doses of 100 and 200 mg/kg i.p. While maximum mortality of 60% occurred at the dose of 600 mg/kg of plant extract.

Fluoxetine, dothiepin and venlafaxine the dose of 30 mg/kg produced maximum reduction in latency of 33, 44 and 37.6% respectively. While there was variable effect of these compounds on PTZ induced mortality in mice. Venlafaxine was the most potent of all tested drugs producing 80% mortality of mice at 30 mg/kg i.p., while at the same dose fluoxetine and dothiepin caused 20 and 40% mortality of mice respectively (Table III).

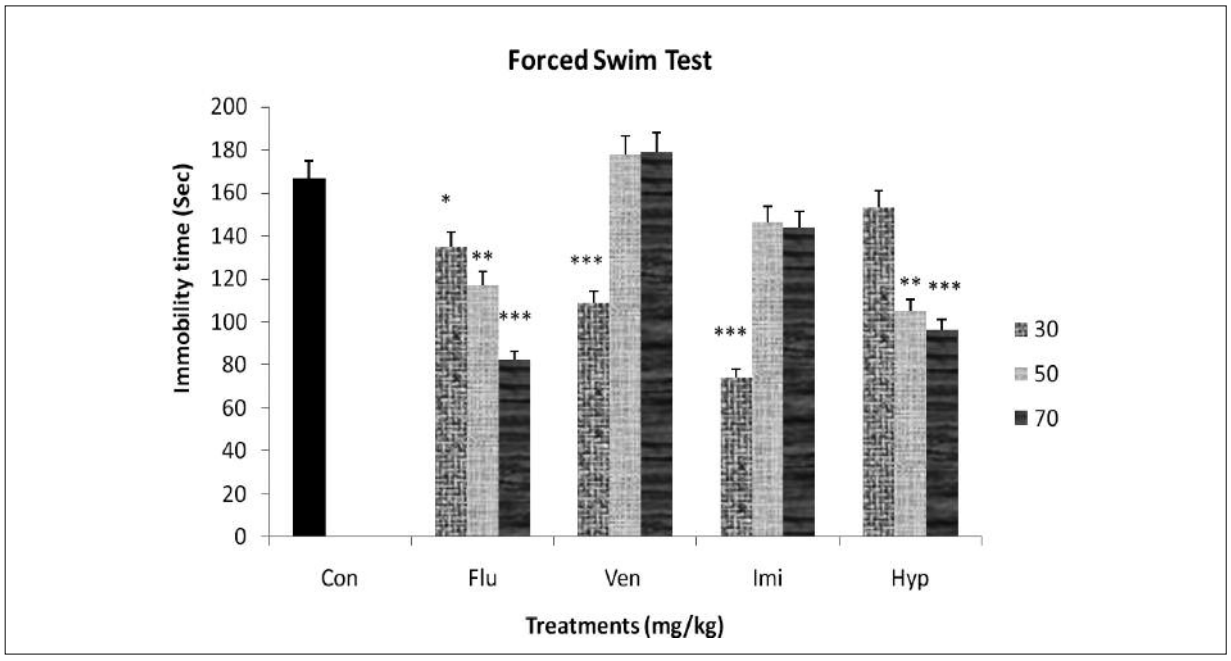


**Figure 2.** Effect of different doses of fluoxetine (Flu), Venlfaxine (Ven), imipramine (Imi) and *H. perforatum* extract (Hyp) on immobility time in rat forced swim test. Rats were given i.p. different doses of the treatments, 1-hour prior to the test session. Control (Con) animals received same volume of vehicle (saline or 10% DMSO). Data represents mean ± SEM (n= 6-10). Asterisks indicate level of significance as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, One way ANOVA followed by the Student-Newman-Keuls multiple comparison test.

### *Locomotor Activity Test*

St. John's Wort (1-30 mg/kg i.p.) decreased locomotor counts in a dose dependent manner. At 1 mg/kg its effect was not significantly different from control group. While, maximum effect (80% reduction) being observed at 30 mg/kg of the extract (Table IV). Similar response was observed with standard antidepressants. As shown in Table IV, the intraperitoneal administration of fluoxetine (30 mg/kg) dothiepin (10 mg/kg) and venlafaxine (50 mg/kg) produced 89, 73, and 62% reduction in locomotor counts of mice respectively.

**Table II.** Effect of *H. perforatum* and standard antidepressant drugs on yohimbine induced lethality in mice.

| Treatment i.p. | Dose (mg/kg) | % Mortality |
|---|---|---|
| Control | – | 0 |
| Yohimbine | 20 | 7 |
| *H. peroforatum* | 10 | 0 |
| | 30 | 33 |
| | 60 | 69 |
| | 100 | 100 |
| Fluoxetine | 10 | 0 |
| | 30 | 25 |
| | 40 | 50 |
| | 50 | 83 |
| | 60 | 100 |
| Dothiepin | 10 | 17 |
| | 20 | 17 |
| | 30 | 50 |
| | 50 | 71 |
| Venlafaxine | 10 | 0 |
| | 20 | 50 |
| | 40 | 100 |

## Discussion

In this study the effects *H. perforatum* were compared with standard antidepressants including fluoxetine, dothiepin, imipramine and venlafaxine, in variety of behavioral animal models.

FST is commonly used for evaluation of antidepressant agents. When a normal animal is forced to swim in a restricted space from which there is no escape, it displays a characteristic immobility posture, reflecting a state of "behavioral despair"[17]. Clinically used antidepressants have been shown to decrease the immobility time in rodents[22]. In the FST *H. perforatum* extract produced a dose dependent decrease in the immobility time in rats and these findings were consistent

**Table III.** Effect of *H. perforatum* and standard antidepressants on pentylenetetrazole induced seizures in mice.

| Treatment | Dose (mg/kg) i.p. | Latency of convulsion (sec) | % mortality | % change in latency. |
|---|---|---|---|---|
| Saline | 10 ml/kg | 180.2 ± 8 | 9 | 0 |
| *H. perforatum* | 50 | 143.8 ± 20* | 20 | -20 |
| | 100 | 101.2 ± 12*** | 20 | -44 |
| | 200 | 102 ± 12.5*** | 40 | -43 |
| | 400 | 126 ± 12** | 100 | -30 |
| | 600 | 181 ± 8.5 | 60 | +1 |
| Fluoxetine | 30 | 120.2 ± 10** | 20 | -33 |
| | 50 | 123.2 ± 11** | 100 | -32 |
| Dothiepin | 30 | 103.2 ± 15*** | 40 | -43 |
| | 50 | 98.4 ± 12.5*** | 60 | -45.3 |
| Venlafaxine | 30 | 112.4 ± 9** | 80 | -37.6 |
| Diazepam | 1 | > 10 min | 0 | 0 |

with earlier reports on crude extracts of the plant[5]. The observed U-shape (biphasic effect) dose-response relationship in FST may be due to mixture of compounds found in the plant extract. The clinically used antidepressants like dothiepin, imipramine (TCA) and fluoxetine (SSRI) also caused significant reduction in immobility time in rats and these results were in accordance with those previously reported for wide range of antidepressants including tricyclic, monoamine oxidase inhibitors and other atypical antidepressants[16,17]. The potency of St. John's Wort extracts in FST was almost equal to fluoxetine and greater than venlafaxine.

It has been reported that various antidepressants including tricyclic antidepressants can reduce the duration of immobility in the swim test by activating catecholaminergic mechanism in the brain[23,24]. Therefore, on the basis of these findings, the attenuation of immobility in FST by St. John's Wort indicates that this effect may have occurred due to increase level of catecholamine, possibly noradrenaline in the brain. However, fluoxetine, at lower doses (30 and 50 mg/kg) did not exhibit significant response in FST; at higher doses (70 mg/kg) significant reduction in immobility was evident. This further suggests that immobility-reducing effect of compounds in FST might be mediated through noradrenergic system and not through 5-HT system, because the high doses of fluoxetine influence the noradrenaline uptake inhibition[25].

Potentiation of yohimbine lethality in mice is also used for the assessment of antidepressant drugs[18]. It has been reported that the potentiation of yohimbine toxicity by the compounds can be predictive of anti-depressant potential[26]. The potentiation of yohimbine lethality has been reported for several antidepressants[16,26]. In this investigation *H. perforatum* extract, fluoxetine, dothiepin and venlafaxine increased yohimbine toxicity. The order of potency was venlafaxine >

**Table IV.** Effect *H. perforatum* and standard antidepressants on the locomotor activity in mice.

| Treatment | Dose (mg/kg) | Locomotor counts | | % change |
|---|---|---|---|---|
| | | Control | Test | |
| St. John,s Wort | 1 | 8158 ± 397 | 6871 ± 297* | -16 |
| | 5 | 7206 ± 655 | 3508 ± 422** | -51 |
| | 10 | 6037 ± 408 | 1799 ± 300*** | -70 |
| | 30 | 7531 ± 510 | 1534 ± 229*** | -80 |
| Fluoxetine | 30 | 5147 ± 392 | 572 ± 60*** | -89 |
| Dothiepin | 10 | 5478 ± 707 | 1477 ± 183*** | -73 |
| | 50 | 5976 ± 365 | 1800 ± 153*** | -70 |
| Venlafaxine | 50 | 5592 ± 640 | 2118 ± 349**** | -62 |
| Vehicle (10 ml/kg) | – | 4763 ± 533 | 5468 ± 404 | +15 |

dothiepin > fluoxetine. The increase in yohimbine induced mortality of mice demonstrated by *H. perforatum* extract and standard antidepressants imply that it is probably due to rise in noradrenaline levels in the brain and peripheral tissues[27,28].

Locomotor activity test is widely employed for evaluation of central nervous system depressant and stimulant agents. It has been reported that various antidepressants like tricyclic antidepressants and monoamine oxidase inhibitors decrease locomotor activity of the mice[16,17]. In our research St. John's Wort extracts and standard antidepressants also reduced the general locomotor behavior of the mice, at dose range that showed profound antidepressant activity in other behavioral tests. Thus, supporting that antidepressant effect of either Hypericum extracts or standard antidepressants was not due to stimulation of the central nervous system[16].

PTZ is known for its action to inhibit γ-aminobutyric acid type-A gamma aminobutyric acid (GABA)-activated channel[29]. It causes convulsions by inhibiting chloride ion channels associated with GABA-A receptors. The potentiation of PTZ induced convulsions has been shown to indicate antidepressant activity of compounds[30]. *H. perforatum* extract, in a way similar to standard antidepressants, increased PTZ induced toxicity and significantly reduced the latency of convulsions. The PTZ induced toxicity has also been shown to increase in the presence of other plant extracts with antidepressants potential such as *Cissampelos sympodialis*[31]. Moreover, standard anti-depressants also reduced the latency of PTZ induced convulsions and their order of potency was dothiepin > venlafaxine > fluoxetine. It is highly unlikely that *H. perforatum* could antagonize the effect of GABA because their activity profile is similar to reference drugs that act via activation of catecholaminergic mechanism in the brain[23,24]. Pretreatment of animals with diazepam, an agonist for benzodiazepine receptors[32,33]. protected the animals against PTZ induced convulsions. No symptoms of central nervous system excitation such as seizures and tremors, salivation, hyperactivity, intermittent jerks were seen in animals treated with diazepam. However, these symptoms were prominent in control group and more intense in animals treated with Hypericum extract or standard antidepressants.

*H. perforatum* also increased PTZ induced mortality of mice. Likewise standard antidepressants enhanced PTZ induced mortality with order of potency as venlafaxine > dothiepin > fluoxetine.

## Conclusions

*H. perforatum* extract demonstrated anti-depressants like properties in FST, resembling that of standard antidepressants. It also potentiated yohimbine and pentylenetetrazole induced lethality, implying that its antidepressant effect is possibly mediated via noradrenaline and GABA neurotransmitters. The effects of *H. perforatum* extract and standard antidepressants show that their antidepressant effect was not due to psychomotor stimulant properties because all the treatments showed significant reduction in locomotor counts of mice.

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