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Original Research Article

Evaluation of antidepressant properties of ethanolic extract of root of *Rubia cordifolia* in Swiss albino mice

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ABSTRACT

Background: Mental health has always been given a prime importance along with physical health by the World Health Organization (WHO). Depression contributes to significant disease burden at global level and is ranked by WHO as the single largest contributor to global disability. Oxidative stress plays an important role in the pathogenesis of many neurological conditions such as depression. *Rubia cordifolia* is known to possess antioxidant properties; hence, this study has been conducted to evaluate the antidepressant activity of Rubia cordifolia root extract in rodent models.

Materials and Methods: Ethanolic extract of *Rubia cordifolia* root powder (EERC) was used for evaluating the antidepressant activity in Swiss albino mice. EERC was used in two doses – 50 mg/kg and 100 mg/kg. Antidepressant activity was evaluated by performing Forced swim test and Tail suspension test with Imipramine (10mg/kg) as the standard drug. The data was analyzed using one way ANOVA (analysis of variance) followed by Tukey Krammer test. P value <0.05 was considered as statistically significant.

Results: Forced swim test and tail suspension test showed significant decrease in the period of immobility by EERC treated groups indicating its antidepressant property. There is no significant difference observed between the two doses of the extract i.e.; 50 mg/kg and 100 mg/kg (inter-group comparison). The period of immobility and period of mobility were not significantly different from the standard group.

Conclusion: This study shows that ethanolic extract of *Rubia cordifolia* root powder possess significant antidepressant properties. However, this is just a preliminary study. Further studies have to be carried out to find out the exact mechanism responsible for antidepressant activity by isolating the active constituents and performing neurotransmitter studies.

1. Introduction

Mental disorders have caused a major disease burden to the world and have increased in the past few years. World Health Organization (WHO) defined psychological health as, "a state of wellbeing in which an individual realizes their own abilities, can withstand the normal stresses of life, can work fruitfully and productively, and can contribute to his or her community".[1] Depression is a psychiatric disorder characterized by tiredness, feelings of guilt, low self-esteem, and sadness, loss of interest, disturbed sleep, disturbed appetite, and poor concentration. Approximately 3.8% of the global population is affected with depression in 2020, including 5.0% among adults and 5.7% among adults older than 60 years. Oxidative stress is one of the key factors which play an important role in the etiopathogenesis of depression. Increased production of hydroxyl and superoxide anions and the inability to destroy them due to deficient antioxidant mechanisms in the brain may produce increased oxidative stress in patients suffering from depression.[2] Even though the available antidepressant medications help to improve the overall quality of life, they

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are associated with a lot of untoward side effects. This is one of the limiting factor and may lead to drug discontinuation, which in turn affects patient's compliance.

Rubia cordifolia Linn is a well-known medicinal plant since ancient times for its medicinal properties. Commonly known as Manjistha or Indian Madder, distributed throughout India, predominantly in the hilly regions. Different parts of this plant have been used for the treatment of problems like gouty arthritis, glandular swellings, recurrent skin infections, leukoderma, gynecological and urinary problems.[3] The roots of *R. cordifolia* are of great medicinal value. *In vitro* and *in vivo* studies have resulted in a wide range of pharmacological actions attributed to the roots of *Rubia cordifolia,* including anti-inflammatory, neuroprotective, hepatoprotective, antidiabetic properties, antibacterial activity, radio-protective activity, antioxidant, and antitumor activities.[4]

From the studies, it has been clear that oxidative stress plays an important role in the pathogenesis of many neurological conditions such as depression. *Rubia cordifolia* is known to possess antioxidant properties, and on literature search, there were no studies on antidepressant activity of this plant. Hence, the current study has been conducted to evaluate the anti-depressant activity of *Rubia cordifolia* root extract in rodent models of depression for validating its effectiveness scientifically.

2. Materials and Methods

Animal Ethics clearance was obtained from the Institutional Animal Ethics Committee before initiating the study (Clearance no: YU/IAEC/17/2019). This study was conducted in the Ethnopharmacology lab, Department of Pharmacology, Yenepoya Medical College, Mangalore, from June 2020 to March 2021.

2.1. Preparation of the extract

Rubia cordifolia root powder (organic) was purchased from Merlion Naturals, Ahmedabad, India. The root powder, which weighed 220g was taken for extraction in soxhlet apparatus using 95% ethanol as a solvent. The temperature was maintained around 60-70^{o}C. The total duration of the extraction process was ten days. The extract obtained was concentrated in the Rotavapour initially and then subsequently in the water bath for 24 hours. The resultant dark red coloured extract weighed 70.6g, and the yield was 32.09%. The extract obtained was stored at 0^{0}C until used. Ethanolic extract of the root of Rubia cordifolia (EERC) was dissolved in distilled water and administered to animals in various doses.[5,6]

2.2. Drugs and chemicals

Distilled water (Vehicle control), Imipramine (standard drug), and Ethanolic extract of *Rubia cordifolia* root powder (EERC) were used in this study.

2.3. Animals

Male and female Swiss albino mice, aged 6-8 weeks, weighing 25-30 gm, were used in this study. Animals were housed under standard conditions in the departmental animal house (Registration no: 347/PO/ReBi-S/Rc-L/01/CPCSEA) with temperature maintained around 24+/-2^{o}C with 12:12 hour light: dark cycle. The animals were given a standard pellet diet and water ad libitum. Animals were acclimatized to the laboratory conditions for seven days before conducting the study. The Animals were handled according to the guidelines of the "Committee for the purpose of Control and Supervision of Experiments on Animals" (CPCSEA). All the experiments were carried out between 7 pm and 9 pm.

2.4. Sample size and study design

For carrying out antidepressant activity by performing forced swimming test (FST) and tail suspension test (TST), a total of 24 Swiss albino mice were randomly divided into 4 groups with 6 animals in each group (either sex). Different sets of animals were used for each experiment. The total number of animals used in the experiment were 24.

1. Group I: Vehicle control – administered distilled water 10 ml/kg, PO
2. Group II: Standard – administered Imipramine 10 mg/kg, PO
3. Group III: Test drug – administered ethanolic extract of R. cordifolia roots ($EERC_1$) 50 mg/kg, PO
4. Group IV: Test drug – administered ethanolic extract of R. cordifolia roots ($EERC_2$) 100 mg/kg, PO

2.5. Experimental procedure

Two doses were taken for conducting the antidepressant study from the literature available i.e., 50 mg/kg and 100 mg/kg.[7] The quantity of the test drug/extract administered was calculated according to the bodyweight of the animals and was administered orally for a period of 14days. On the 14th day, after one hour of drug administration, animals were tested for antidepressant activity by performing forced swimming test (FST). After that, a washout period of 6 weeks has been given. After the washout period, again, test drug/extract was administered to the animals for a period of 14 days. On the 14th day, after one hour of drug administration, animals were tested for antidepressant activity by performing tail suspension test (TST).

2.6. Forced swimming test (FST)

Mice were housed in individual polypropylene cages one day before the experiment. Each mouse was brought into a vertical plexiglass cylinder (40 cm x 18cm and containing

15 cm of water at 25^{o}C) and was observed for 6 minutes. The evaluation was initiated after 2 minutes. Initially, mice were hyperactive, vigorously swimming in circles, trying to climb the wall, or diving to the bottom. After 2-3 min this activity subsided, and there was a period of immobility or floatation of increased duration. Duration of immobility was noted for each mouse. The antidepressant activity was represented by an increase in the duration of mobility or a decrease in the duration of immobility. After the test, animals were allowed to dry and returned to their home cages.[8]

2.7. Tail suspension test

Each mouse was suspended upside down 50 cm above the ground with the help of a stand and using an adhesive tape placed approximately 1 cm from the tip of the tail. They were observed for 5 minutes. Mice were considered immobile when it was hanging freely without making any movements. Duration of immobility was noted for each Mouse. The antidepressant activity was represented by an increase in the duration of mobility or a decrease in the duration of immobility. After the test, the animals were returned to their home cages.[8]

2.8. Statistical analysis

Observations made in FST and TST were compiled and tabulated using the statistical software, GraphPad, InStat. Results were represented as Mean ± SEM (Standard Error of Mean). One-way analysis of variance (ANOVA) was used for simultaneous comparison of groups. For those variables that were significant in ANOVA, multiple comparison between the groups were made with Tukey Kramer Test at P = 0.05. The statistical analysis was carried out using the software SPSS v24.0. P-value $\leq$ 0.05 was considered as significant, P-value $\leq$ 0.01 was considered as highly significant, and P-value $\leq$ 0.001 was considered as very highly significant.[9]

3. Results

3.1. Forced swimming test (FST)

EERC showed significant increase in the period of mobility/decrease in the immobility time when compared to the control group. There is no significant difference observed between the two doses of the extract i.e., 50 mg/kg and 100 mg/kg (inter-group comparison). The period of immobility and period of mobility were not significantly different from the standard group.(Table 1)

3.2. Tail suspension test (TST)

EERC showed significant increase in the period of mobility/decrease in the immobility time when compared to the control group. There is no significant difference observed between the two doses of the extract i.e., 50 mg/kg and 100 mg/kg (inter-group comparison). The period of immobility and period of mobility were not significantly different from the standard group.(Table 2)

4. Discussion

The present study is performed to evaluate the antidepressant activity of ethanolic extract of *Rubia cordifolia* root in experimental animal models. From Tables 1 and 2, we can observe that the extract showed a significant decrease in immobility in behavioral models of mice after administering the extract for a period of 14 days.

Depression is a chronic psychiatric condition characterized by low mood, loss of appetite, irritability, and difficulty in focusing. Many of the depressed patients present with suicidal ideation.[10] Various hypotheses are explaining the pathophysiology of depression, and the monoamine hypothesis is the most common and widely accepted among them. It is well said that depletion of monoamines leads to depression.[11] The hypothesis describing the association between serotonin levels and depression is almost half a century old. It is undoubtedly known that serotonin depletion plays an important role in the pathophysiology of depression. This is again substantiated by the fact that Selective Serotonin Reuptake Inhibitors (SSRIs), which increase serotonin levels play a vital role in the pharmacotherapy of depression.[12] Literature also suggests that oxidative stress plays an important role in the pathophysiology of depression and associated neuropsychiatric diseases. Evidence generated over the past twenty years indicates that the imbalance between oxidative stress and antioxidant defense mechanisms is a major pathogenic cause of depression. Major depressive disorder (MDD) is associated with a lowered concentration of antioxidants in plasma, and their imbalance leads to an increase in the activity of pro-inflammatory pathways, and other apoptotic mediators leading to neurodegeneration. Currently available antidepressants not only affect the levels of neurotransmitters but also modify the imbalance between oxidant and antioxidant substances.[13]

Currently used conventional antidepressant drugs act mainly on the monoaminergic system by increasing their levels. Although these drugs increase the monoamine levels and improve depressive symptoms, they also cause numerous adverse effects like dry mouth, weight gain, sweating, etc. and, also the clinical response is not obtained immediately.[14] Therefore, there is a need for an antidepressant drug with better clinical profile.

Various animal models are available for demonstrating the antidepressant activity of potential drugs. In this study, forced swim test (FST) and tail suspension test (TST) are the two animal models used to evaluate the antidepressant activity of EERC. In both the models, period of immobility was the parameter used to assess the antidepressant activity.

Table 1: Antidepressant activity of EERC-Forced swimming test

SI No.	Group	Period of mobility (seconds)	Period of immobility (seconds)
I	Control	108.67±5.989	131.33±5.989
II	Imipramine	182.33±7.421^a	57.67±7.421^a
III	$EERC_1$	165.50±5.788^b	74.50±5.788^b
IV	$EERC_2$	166.00±5.550^c	74.00±5.550^c

$EERC_1$ – ethanolic extract of *Rubia cordifolia* root 50 mg/kg $EERC_2$ – ethanolic extract of *Rubia cordifolia* root 100 mg/kg One-way ANOVA followed by Tukey Krammer test ^{a}P-value <0.001 – very highly significant compared to Group I ^{b}P-value <0.01 – highly significant compared to Group I ^{c}P-value <0.01 – highly significant compared to Group I No significant difference observed between $EERC_1$ & $EERC_2$

Table 2: Antidepressant activity of EERC-Tail suspension test

SI No.	Group	Period of mobility (seconds)	Period of immobility (seconds)
I	Control	136.83±7.278	163.17±7.278
II	Imipramine	199.00±7.071^a	101.00±7.071^a
III	$EERC_1$	178.50±5.394^b	121.50±5.394^b
IV	$EERC_2$	180.83±5.115^c	119.17±5.115^c

$EERC_1$ – ethanolic extract of *Rubia cordifolia* root 50 mg/kg $EERC_2$ – ethanolic extract of *Rubia cordifolia* root 100 mg/kg One-way ANOVA followed by Tukey Krammer test ^{a}P-value <0.001 – very highly significant compared to Group I ^{b}P-value <0.01 – highly significant compared to Group I ^{c}P-value <0.01 – highly significant compared to Group I No significant difference observed between $EERC_1$ & $EERC_2$

Decrease in the period of immobility was considered as an indicator of antidepressant activity.

Currently, forced swim test (FST) is considered as one of the reliable models for testing potential antidepressant drugs. This test has the advantage of differentiating antidepressants from neuroleptics and anti-anxiety drugs. Also, it provides a platform for assessing various neurobiological and genetic mechanisms influencing stress and antidepressant responses. Forced swim test is highly sensitive to monoaminergic transmission.[15] Various literature suggest that drugs that influence serotonin transmission increase the swimming behaviour in rodents.[16] EERC showed a decrease in the period of immobility compared to the control, which was statistically significant, suggesting the antidepressant action.

Tail suspension test (TST) is another important model for evaluating the activity of various potential antidepressant drugs. This test is considered as 'dry version' of forced swim test, because the parameter assessed in tail suspension test is identical to forced swim test although the procedure done is exclusively different. This test is also specific to antidepressants as it differentiates antidepressants from other drugs used for psychosis, anxiety, and attention deficit hyperactivity disorder (ADHD). Previous studies shows that there is genetic background in the behavioral responses observed in the tail suspension test.[17] EERC showed a significant decrease in the period of immobility compared to control group. Phytochemical studies on *Rubia cordifolia* showed the presence of phytochemicals like anthraquinones which have potential antioxidant activities in animal models. Previous studies have shown that *Rubia cordifolia* was found to be effective in increasing the antioxidant levels, such as GSH, and has a role in the expression of the gamma-glutamyl cysteine ligase and Cu-Zn superoxide dismutase (SOD) genes. RC root extract also exhibited a strong free radical scavenging properties against reactive oxygen species and reactive nitrogen species.[18,19] Thus, the observed antidepressant activity of EERC could be due to the change in the oxidant and antioxidant parameters in the mouse brain, which can be attributed to the presence of phytochemicals such as anthraquinones. Further studies are needed to be done by estimating the neurotransmitter levels as well as antioxidant levels to prove the exact mechanism contributing to the antidepressant action of EERC.

5. Conclusion

From this study, it can be concluded that EERC (ethanolic extract of *Rubia cordifolia* root powder) shows a significant antidepressant activity in the doses of 50 mg/kg and 100 mg/kg, although there was no dose-dependent action seen between the two doses. Previous phytochemical studies have shown that anthraquinones are the major constituent of *Rubia cordifolia* root, which has antioxidant properties. Therefore, it can be presumed that the antidepressant action of the root of *Rubia cordifolia* is by elevating the antioxidant levels in the brain. However, this is just a preliminary study. Further studies have to be carried out to determine the exact mechanism responsible for antidepressant activity by isolating the active constituents and performing neurotransmitter studies.

6. Abbreviations

1. ADHD - Attention deficit hyperactivity disorder
2. ANOVA - Analysis of Variance
3. CPCSEA - Committee for the Purpose of Control and Supervision of Experiments on Animals
4. EERC - Ethanolic Extract of root of *Rubia cordifolia*
5. FST - Forced swimming test
6. GSH - Glutathione
7. IAEC - Institutional Animal Ethics Committee
8. MDD - Major depressive disorder
9. RC - *Rubia cordifolia*
10. SEM - Standard error of mean
11. SOD - Superoxide dismutase
12. SSRI - Selective serotonin reuptake inhibitors
13. TST - Tail suspension test
14. WHO - World Health Organization

7. Source of Funding

None.

8. Conflict of Interest

None.

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