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PHARMACOLOGICAL EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF *ROSA CENTIFOLIA* PETALS IN MICE

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ABSTRACT

To study the antidepressant activity with aqueous extract of Rosa *centifolia.* The extract was primarily subjected for preliminary phytochemical investigation and for Maximum Tolerance Dose (MTD). Antidepressant activity was evaluated in various animal models like Forced swim test, Tail suspension test, Apomorphine induced hypothermia and 5-HTP potentiation of head twitches in mice. The aqueous extract of *Rosa centifolia* was positively identified with carbohydrates, tannins, proteins, amino acids, alkaloids, flavonoids, flavanone, glycosides and phenolic compounds. The extract was subjected for maximum tolerance dose upto the dose level of 2000mg/kg has not produced any mortality. The extract of *Rosa centifolia* showed significant antidepressant activity at high dose (100 mg/kg) in Tail suspension test, Forced swim test and Potentiation of 5-HTP induced head twitches. The extract didn't antagonized the hypothermia induced by apomorphine.

Keywords: *Rosa centifolia,* anti-depressant activity, forced swim, Apomorphine induced hypothermia, & 5-HTP potentiation of head twitches.

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INTRODUCTION

Depression is among the leading causes of disability worldwide and is common, affecting about 121 million people worldwide. It is a state of low mood and aversion to activity that can affect person's thoughts, behavior, feelings and physical well-being. It may include feelings of sadness, anxiety, emptiness, hopelessness, worthlessness, guilt, irritability, or restlessness. Depression is associated with changes in substances in the brain (neurotransmitters) that help nerve cells communicate, such as serotonin, dopamine and norepinephrine. It is quite common in women than men. Depression can affect people of any age, including children. Studies have shown that about 4% of children aged 5-16 are affected by depression.

Depression is caused by four major biological factors include genetic factors, biochemical factors includes abnormalities in the delivery of certain key neurotransmitters, and alterations in hormonal flow, environmental factors includes stressful life changes, failing a class, illness or death in the family, or parents divorcing, childhood events, such as abuse or neglect, death of a friend or relative, or loss of a job, social isolation, medical conditions such as hypothyroidism, medications (such as sedatives and high blood pressure medications), cancer, major illness, or prolonged pain and sleeping problems, stroke, Parkinson's disease, or multiple sclerosis [1,2].

The symptoms of depression varies with individuals based on severity and also varies over time, which includes feeling sad, blue, hopeless, loss of interest or pleasure in ordinary activities once enjoyed, waking up early in the morning or sleeping too much, change in eating habits, being anxious, pessimistic, or worried, feeling guilty, helpless, or worthless, difficulty concentrating, fatigue and loss of energy, appearing slowed or agitated [3].

There are number of drugs to treat the depression where the treatment is not satisfactory often due to severe adverse effect of drugs and thus demand for the search for new and safer one. Presently there is a resurgence of interest among health care professionals for potent and safe antidepressant drug. Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Thus, it is worthwhile to look for antidepressants from plants with proven advantage and favourable benefit-to-risk ratio. A number of medicinal plants and medicines derived from these plants have shown antidepressant properties by virtue of their medicinal constituents. The present review is focused on the medicinal plants and plant-based formulations having antidepressant activity in animal studies [4].

Rosa centifolia commonly known as Red rose or Pink rose belongs to the family Rosaceae. It consists of volatile oil, tannic acid, coloring matter, saccharine matter, mineral salts, and salts of mallic and tartaric acids, etc. In addition to substantial proportions of vitamin C. Rose petals are used for rejuvenating and prove to be a tonic. They are used to treat internal asthma, high blood pressure, bronchitis, slow circulation, diarrhoea, dysmenorrhoea (painful

menstruation), cough, fever and fluid retention, indigestion, insomnia, palpitation, stress and urinary tract infections, depression, fatigue and comfort tetchiness etc [5].

In the present study, we have chosen a plant *Rosa centifolia* that is a popular medicinal agent in ethnic medicine. But scientific data regarding the use of *Rosa centifolia* for antidepressant activity is not available. Hence we have selected this plant to scientifically evaluate its antidepressant activity.

MATERIALS AND METHODS

MATERIALS

Apomorphine, Desipramine, 5-Hydroxy Tryptophan, Pargyline hydrochloride were obtained from Sigma life sciences,Bangalore. Fluoxetine (Fludac,Cadila), Glacial acetic acid, NaOH (Reagent Lab Systems Hyderabad), Electronic Thermometer (UGO BASILE), Narrow glass cylinder(13 cm in diameter × 24 cm high (Fabricated by Natco)

Animals

Albino mice of either sex weighing between 18-25 gm were used in this study. All the animals were acclimatized for 7 days in quarantine room and housed in groups of six under standard husbandry conditions like room temperature 23 ± 2^0C, relative humidity 45-55% and light/ dark cycle of 12 hours at animal House, NATCO Research Centre, Sanathnagar.

All the animals were fed with synthetic standard diet (National Institute for Nutrition, Hubsiguda, and Hyderabad) and water was supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of NATCO Research Centre. All animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA. CPCSEA Reg No: 1236/C/08/CPCSEA.

All the animals were fasted 3hrs prior to the oral administration of vehicle/standard/test compounds during the experiment. All experiments were carried out during the light period (9:00 to 17:00 h) to avoid circadian rhythm.

Preparation of Extract

The dried petals were crushed into fine particles (powder) using a mixer. The powdered material (500 g) was soaked in distilled water and subjected to maceration. Extract obtained was passed filtered, evaporated and dried in desiccators [6].

Preliminary Phytochemical Screening

The preliminary phytochemical investigations will be carried out with the aqueous extract of *Rosa centifolia* for qualitative identification of phytoconstituents.

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EXPERIMENTAL DESIGN

Determination of Acute Toxicity (LD_{50})

The acute toxicity of aqueous extract of *Rosa centifolia* was determined by using female albino mice (18-25g) those maintained under standard husbandry conditions. The animals were fasted for 3 hrs prior to the experiment, up and down procedure (OECD guideline no. 425) of CPCSEA was adopted for toxicity studies [7]. Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (Short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. The LD_{50} of the test extract was calculated using AOT 425 software provided by environmental protection agency, USA [8, 9]. From the LD_{50} dose $1/40^{th}$ and $1/20^{th}$ doses are to be selected and considered as low and high dose respectively.

Grouping of animals and treatment schedule

Male albino mice (18-25g) were divided into four groups each consisting of 5 animals. For Forced swim test, Tail suspension test, 5-HTP potentiation of head twitches in mice, Group I- Normal control, Group II- Standard (Flupxetine25mg/kg, p.o), Group III –Low dose of AERC (50mg/kg, p.o), Group IV – High dose of AERC (100mg/kg, p.o). Incase of Apomorphine induced hypothermia Group II- Standard (Desipramine 20mg/kg p.o) and remaining all groups was same as mentioned above.

Methods

Forced Swim Test

Experiment was carried out in narrow glass cylinder (13 cm in diameter × 24 cm high) containing water (25°C) to a depth of 10 cm, from which they cannot escape. All the animals were fasted for 3hrs prior to the oral administration. All the animals were administered with vehicle/standard/test compound by orally to the respective groups. Thirty minutes later, the animals were subjected to swim. Immobility time was recorded at 60, 120, 240 min after oral administration for 6 minutes; the first two minutes the animal was allowed to adjust to the new conditions; for the next four minutes the immobility time that alternated with conditions of enhanced motor activity was measured with a stopwatch. Immobility time is the time during which the animal floated on the surface with front paws together and made only those movements which were necessary to keep afloat [10-14].

Tail Suspension Test

All the animals were fasted for 3hrs prior to the oral administration of vehicle/standard/test compounds. The test and standard compounds were administered orally 60 minutes prior to testing. The mice were suspended on the edge of a shelf 58cm above the

table top by adhesive tape placed approx. 1cm from the tip of tail. The duration of immobility was recorded for the period of 6minutes by using stopwatch. After the initial period of vigorous motor activity, the mice would become still. Mouse was considered immobile when they hang passively and completely motionless [15, 16].

Apomorphine Induced Hypothermia

All the animals were fasted for 3hrs prior to oral administration of vehicle/standard/test compounds. All the animals were administred with vehicle/standard/test compound by orally to the respective groups. One hour after oral administration, 16mg/kg apomorphine was injected s.c. to the animals. The rectal temperature of each mouse was measured by an electronic thermometer prior and 10, 20, 30, 60 and 120 minutes after apomorphine administration. During the entire experiment, animals were housed in groups at room temperature [17].

5-HT Potentiation of Head Twitches In Mice

The animals were fasted for 3hrs prior to oral administration. Animals were administered with vehicle/standard/test compounds orally to the respective groups. After 60 minutes, animals were injected with 75mg/kg pargyline Hydrochloride, subcutaneously. 90 minutes after pargyline, the animals were injected with 5-HT, 10mg/kg i.p. The number of head twitches and behavioural parameters like escape tendency, hind limb abduction,tremors,fore limb clonus and lardosis were calculated for half an hour [18].

The scoring of various behavioural parameters is as follows

Escape tendency	0 or 1
Hind limb abduction	0-2
Tremors	0-4
Fore limb clonus	0-2
Lardosis	0-2

Statistical Analysis

Results were presented as means ± SEM .The data was subjected for statistical analysis by One- way Analysis of Variance (ANOVA) followed by Dunnet's **'t'** test using instat software. P < 0.05*, 0.01** and 0.001*** were considered as significant.

RESULTS

PRELIMINARY PHYTOCHEMICAL SCREENING

The percentage yield of aqueous extract of *Rosa centifolia* petals was 17.6%. The aqueous extract of *Rosa centifolia* was subjected for phytochemical screening and found that carbohydrates, alkaloids, proteins, amino acids, flavonoids, flavanone, glycosides, tannins and phenolic compounds were present. The results were as follows

ASSESSMENT OF ANTIDEPRESSANT ACTIVITY

TAIL SUSPENSION TEST (TST)

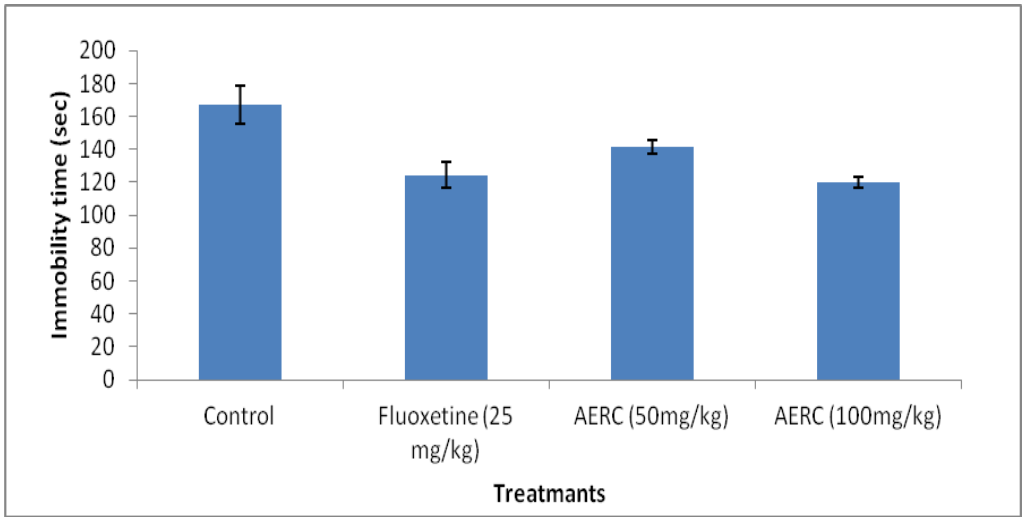
As seen in Table No. 1 and Figure 1, it was observed that animals treated with Fluoxetine (25 mg/kg;p.o) & test dose 100 mg/kg of AERC showed significant decrease in their immobility time but not 50 mg/kg of AERC. Percentage inhibition of immobility time of Fluoxetine, 50mg/kg & 100 mg/kg AERC were 25.7%, 15.5% & 28.34% respectively.

Table No. 1: Effect of *Rosa centifolia* on immobility time in Tail suspension test

S.NO	TREATMENT	IMMOBILITY TIME (SEC)	% INHIBITION OF IMMOBILITY
1	Control	167.2 ± 11.33	-
2	Fluoxetine (25 mg/kg)	124.2 ± 7.82**	25.7
3	AERC (50mg/kg)	141.2 ± 4.16^{ns}	15.5
4	AERC (100mg/kg)	119.8 ± 3.35**	28.34

n=5 in each group. Data is expressed as mean ± SEM. Significance at *P* <0.05*, *P* <0.01** and ns-non significant vs. control group.

Figure No.1: Effect of *Rosa centifolia* on immobility time in Tail suspension test



FORCED SWIM TEST (FST)

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As seen in Table No. 2 & 3 and Figure 2, it was observed that animals treated with Fluoxetine (25 mg/kg;p.o) and test dose 100 mg/kg of AERC showed significant decrease in their immobility time but not 50 mg/kg of AERC. Percentage inhibition of immobility time of Fluoxetine, 50 mg/kg & 100 mg/kg AERC were 48.2%, 22.8% & 36.3% respectively at 120 min.

Table No.2: Effect of *Rosa centifolia* on immobility time in Forced swim test

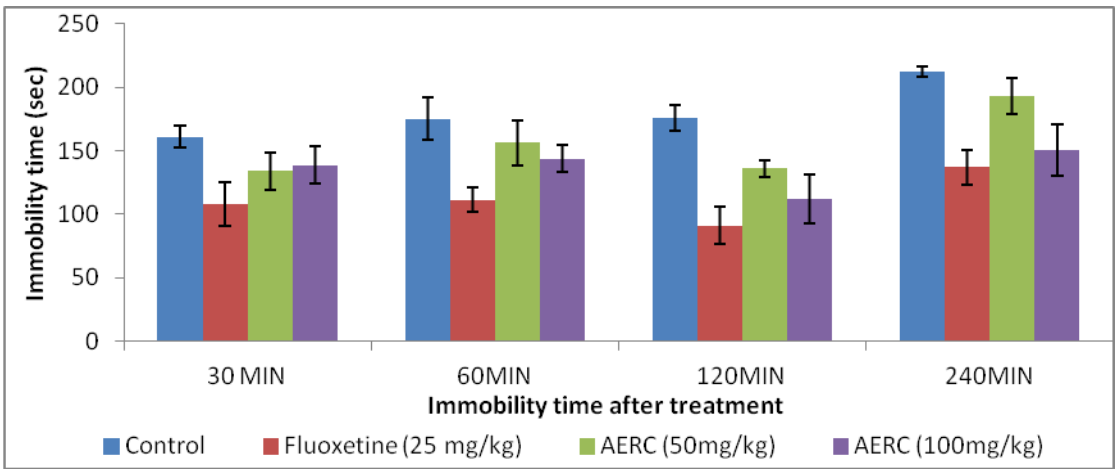
S.NO	TREATMENT	IMMOBILITY TIME AFTER TREATMENT(sec)			
		30 Mins	60Mins	120Mins	240Mins
1	Control	161 ± 8.8	175.2 ± 16.4	176 ± 9.9	211.8 ± 4.1
2	Fluoxetine (25 mg/kg)	107.8 ± 16.9	111.2 ± 9.5	91.2 ± 14.26	137.2 ± 13.6
3	AERC (50 mg/kg)	133.8 ± 14.4 ^{ns}	156.2 ±1 7.5 ^{ns}	135.8 ± 6.25 ^{ns}	193.4 ± 14.08^{ns}
4	AERC (100 mg/kg)	138.8 ± 15.12 ^{ns}	143.4 ± 10.61 ^{ns}	112 ± 19.0**	150.6 ± 20.17*

n=5 in each group. Data is expressed as mean ± SEM. Significance at P <0.05*, P <0.01** and ns-non significant vs. control group.

Table No. 3: Percentage inhibition of immobility time in Forced swim test

S.NO	TREATMENT	% inhibition of immobility time			
		30Mins	60Mins	120Mins	240Mins
1	Fluoxetine (25 mg/kg)	33.04	36.5	48.2	35.2
2	AERC (50 mg/kg)	16.9	10.8	22.8	8.7
3	AERC (100 mg/kg)	13.7	18.1	36.3	28.9

Figure No.2: Effect of *Rosa centifolia* on immobility time in Forced swim test



APOMORPHINE INDUCED HYPOTHERMIA

As seen in Table No. 4, 5 &6 and Figure 3 & 4, it was observed that animals treated with Desipramine (20mg/kg;p.o) but not the two test doses of AERC (50, and 100 mg/kg, p.o)

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significantly antaginised the hypothermia induced by apomorphine when compared to control group.

TableNo.4: Effect of *Rosa centifolia* on degree of hypothermia in Apomorphine induced hypothermia

S.NO	TREATMENT	10Mins	20Mins	30Mins	60Mins	120 Mins
1	Control	-1.92	-2.72	-3.34	-4.36	-4.42
2	Desipramine (20 mg/kg)	-1.41	-0.53	-0.11	-1.67	-1.61
3	AERC (50 mg/kg)	-5.1	-5.48	-5.52	-6.42	-3.08
4	AERC (100 mg/kg)	-4.7	-5.36	-5.66	-5.44	-2.92

Table No. 5 : Percentage inhibition of temperature in Apomorphine induced hypothermia

S.NO	TREATMENT	10Mins	20Mins	30Mins	60Mins	120 Mins
1	Desipramine (20 mg/kg)	1.90	6.07	8.90	7.7	8.05
2	AERC (50 mg/kg)	7.44	7.58	6.9	3.59	3.65
3	AERC (100 mg/kg)	8.82	8.22	6.88	6.85	2.87

Figure No. 3: Effect of *Rosa centifolia* on degree of hypothermia in Apomorphine induced hypothermia

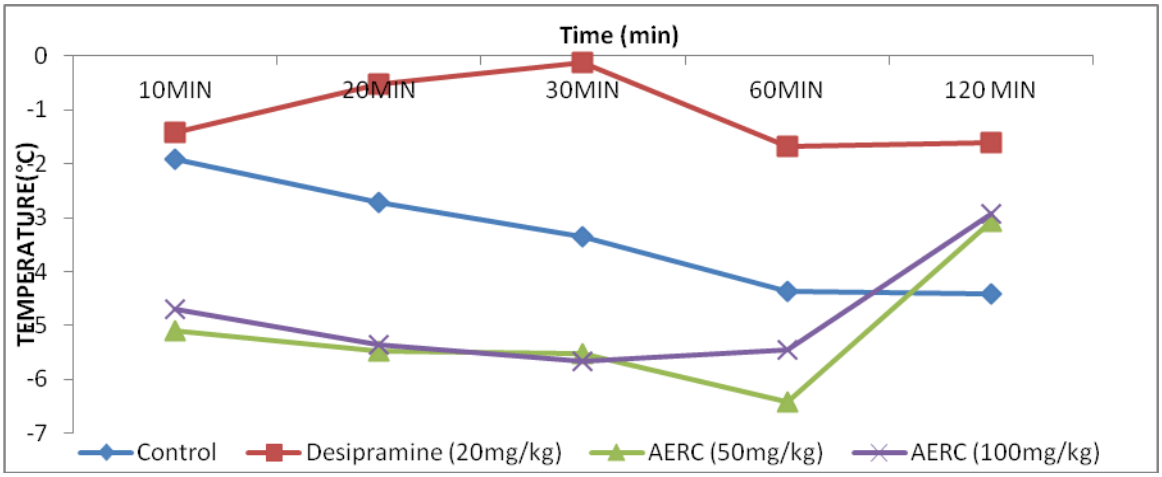

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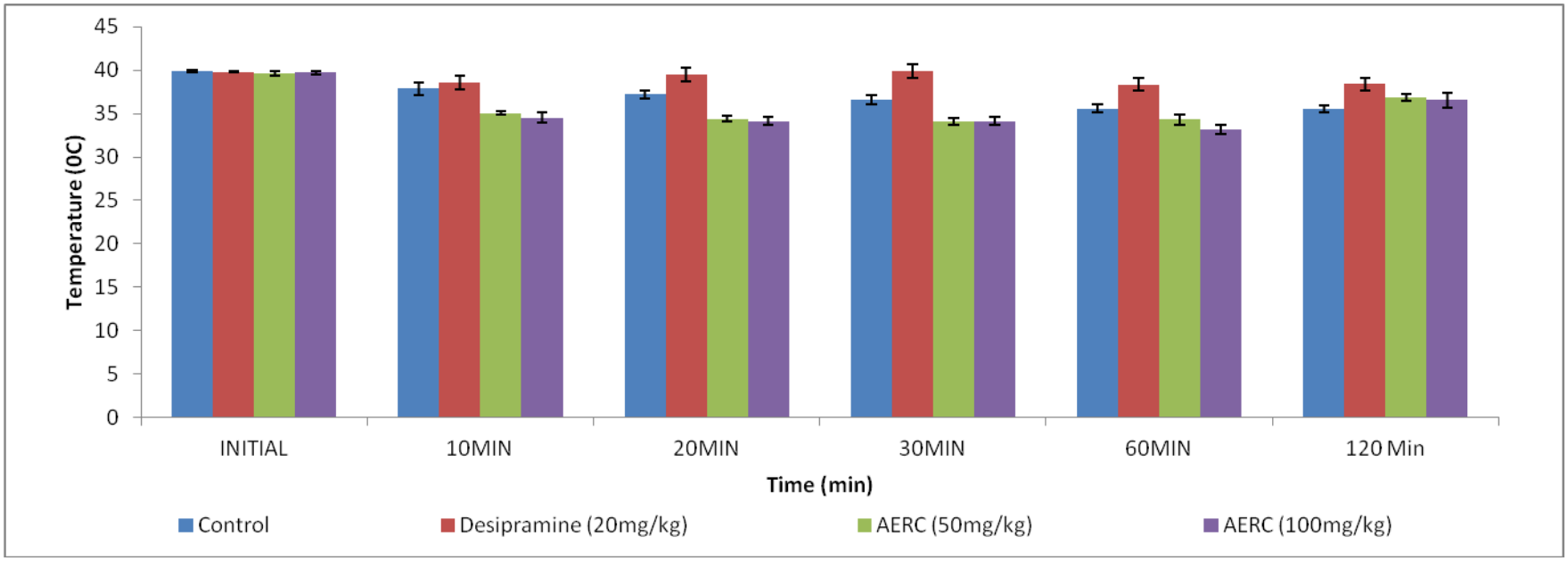
Table No. 6: Effect of *Rosa centifolia* on temperature in Apomorphine induced hypothermia

S.NO	TREATMENT	BASAL TEMP	TEMPERATURE AFTER TREATMENT(^{0}C)				
			10Mins	20Mins	30Mins	60Mins	120 Mins
1	Control	39.88 ± 0.16	37.86 ± 0.69	37.2 ± 0.47	36.62 ± 0.49	35.58 ± 0.43	35.52 ± 0.44
2	Desipramine (20 mg/kg)	39.78 ± 0.08	38.58 ± 0.80	39.46 ± 0.80*	39.88 ± 0.75**	38.32 ± 0.73*	38.38 ± 0.71*
3	AERC (50 mg/kg)	39.62 ± 0.21	35.04 ± 0.21^{ns}	34.38 ± 0.34^{ns}	34.08 ± 0.41^{ns}	34.3 ± 0.55^{ns}	36.82 ± 0.37^{ns}
4	AERC (100 mg/kg)	39.74 ± 0.21	34.52 ± 0.60^{ns}	34.14 ± 0.45^{ns}	34.1 ± 0.46^{ns}	33.14 ± 0.54^{ns}	36.54 ± 0.89^{ns}

n=5 in each group. Data is expressed as mean ± SEM. Significance at *P* <0.05*, *P* <0.01** and ns-non significant vs. control group.

Figure No. 4: Effect of *Rosa centifolia* on temperature in Apomorphine induced hypothermia



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5-HTP POTENTIATION OF HEAD TWITCHES

As seen in Table No. 7 &8 and Figure 5 &6, it was observed that animals treated with Fluoxetine (25 mg/kg;p.o) and test dose 50 mg/kg but not 100 mg/kg of AERC showed significant increase in number of head twitches compared to control group. The other behavioral parameters like escaping tendency, fore limb clonus, hind limb abduction, tremors and lardosis did not show significance in fluoxetine, AERC (50 & 100 mg/kg;p.o) treated groups compared to control groups as shown in Table no.14 & Fig no.14, 15.

Table No.7: Effect of *Rosa centifolia* on the number of head twitches in 5-HTP induced head twitches in mice

S.NO	TREATMENT	NUMBER OF HEAD TWITCHES	PERCENTAGE POTENTIATION
1	Control	8.2 ± 0.37	-
2	Fluoxetine(25 mg/kg)	18.6 ± 1.030^{ns}	126.8
3	AERC (50 mg/kg)	10.6 ± 1.077^{ns}	29.2
4	AERC (100 mg/kg)	13.8 ± 0.73^{ns}	68.2

n=5 in each group. Data is expressed as mean ± SEM. Significance at *P* <0.05*, *P* <0.01** and ns-non significant vs. control group.

Figure No. 5: Effect of *Rosa centifolia* on the number of head twitches in 5-HTP induced head twitches in mice

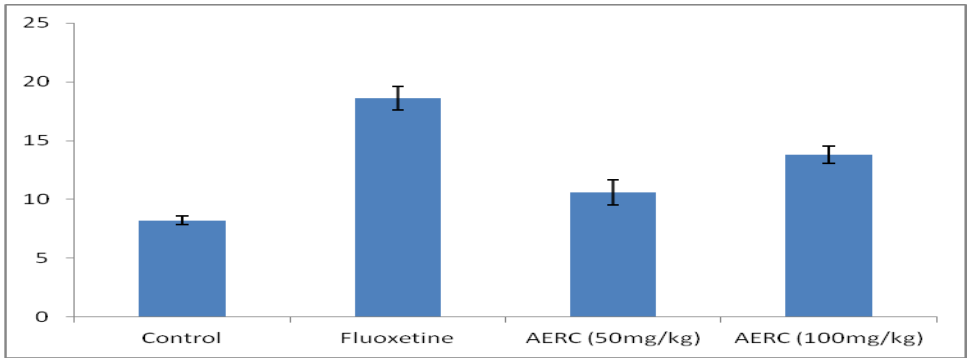

Table No. 8: Effect of *Rosa centifolia* on behavioral parameters in 5-HTP induced head twitches in mice

S.NO	TREATMENT	SCORING OF BEHAVIORAL PARAMETERS	PERCENTAGE POTENTIATION
1	Control	3.56 ± 0.10	-
2	Fluoxetine (25 mg/kg)	5.6 ± 0.09^{ns}	57.3
3	AERC (50 mg/kg)	4.38 ± 0.05^{ns}	23.03
4	AERC (100 mg/kg)	5.24 ± 0.05^{ns}	47.19

n=5 in each group. Data is expressed as mean ± SEM. Significance at *P* <0.05*, *P* <0.01** and ns-non significant vs. control group.

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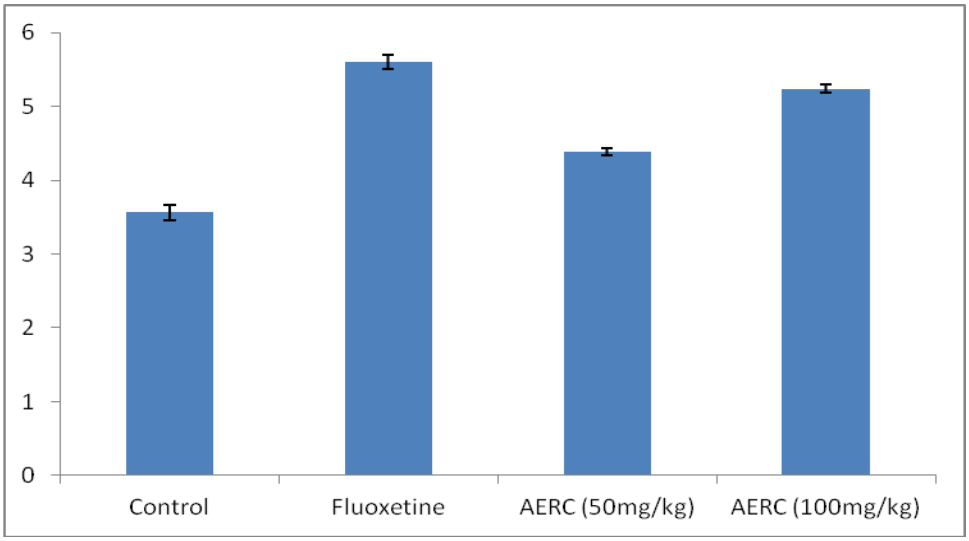
Fig No. 6: Effect of *Rosa centifolia* on various parameters in 5-HTP induced head twitches in mice



DISCUSSION

Depression is a common, debilitating, life-threatening illness with a high incidence associated with lot of morbidity. Hence, it is very important to address this problem and find effective remedies. Even though several drugs are available, they associated with side effects including SSRIs [17] Therefore, there is an urgent need for alternative medications for the control of depression-related disorders.

The present work was subjected to investigation for the evaluation of the anti depressant activity of aqueous extract of *Rosa centifolia* petals in animal models.

In phytochemical screening, the extract showed the presence of carbohydrates, alkaloids, tannins, proteins, amino acids, flavonoids, flavanone, glycosides and phenolic compounds.

In Acute Oral Toxicity study, AERC did not show any lethal effect even up to the doses of 2000mg/kg, p.o and complete absorption of drug through GIT was observed and thus the test doses of 50 & 100mg/kg, p.o were used for evaluation of antidepressant activity.

To assess antidepressant activity, various animal models like Tail Suspension Test, Forced Swim Test, Apomorphine induced hypothermia and 5-HTP Potentiation of induced head twitches in mice were used.

Forced swim test and Tail suspension test are quite sensitive and relatively specific to all major classes of anti depressants and used to evaluate the potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress such as FST & TST are thought to reflect behavioral despair, which reflects depression.

antidepressants reduce the immobility time that mice display after active and unsuccessful attempts to escape when suspended by tail [19,20]. In these tests, animals treated with test dose 100 mg/kg of AERC showed significant decrease in their immobility time but not 50 mg/kg of AERC indicating significant antidepressant like effect were found to be comparable with Fluoxetine (25 mg/kg, p.o).

For the assessment of mechanism of action of AERC, antagonism of apomorphine induced hypothermia and 5-HTP induced head twitches model were used to know AERC acting through noradrenaline or 5-HT.

Antagonism against Apomorphine induced hypothermia can be regarded as a hint for antidepressant activity for noradrenaline uptake. Compounds with a marked noradrenaline or dopaminergic components antagonize apomorphine induced hypothermia but not antidepressants acting mainly through serotonergic system [21]. In this model, both doses of AERC (50 & 100 mg/kg) did not antagonize the apomorphine induced hypothermia, representing that the antidepressant activity exhibited by AERC may be through serotonergic system but not via adrenergic or dopaminergic systems.

5-HT Potentiation of induced head twitches is used for evaluation of antidepressants acting via serotonergic system. 5-HTP is used as the precursor of serotonin, which causes head-twitches. Antidepressant agents acting through this system, potentiate serotonin effects by blocking re-uptake of serotonin [22]. The results showed that AERC (100mg/kg, p.o.) significantly increased cumulative number of 5-HTP induced head twitches and other behavioral parameters. Whereas at higher dose diminishes the antidepressant activity. This suggests that the antidepressant effect of AERC is related to the potentiation of brain serotonergic neurotransmission (reuptake).

The extract of *Rosa centifolia* showed significant antidepressant activity at higher dose (100 mg/kg) in Tail suspension test, Forced swim test and 5-HTP Potentiation of induced head twitches. The extract didn't antagonize the hypothermia induced by apomorphine. This demonstrates antidepressant activity exhibited by AERC is acting via serotonergic system (reuptake inhibition) and not through noradrenergic system.

Therefore all these observations showed that *Rosa centifolia* posses significantly antidepressant activity at higher dose of (100 mg/kg), p.o.

CONCLUSION

The results obtained from these experimental models clearly confirmed the antidepressant activity of AERC in mice. Phytoconstituents like flavanoids were found in aqueous extract which are also reported for their antidepressant activity. So these active components might be responsible for antidepressant effect of AERC.

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