ANTIDEPRESSANT ACTIVITY AND LCMS ANALYSIS OF *CRINUM DEFIXUM*

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Abstract

Context: Although Crinum defixum Linn.(amaryllidaceae), is a bulbous herb which has a wide geographical distribution in India., possesses diverse pharmacological activities in animals, little is known about its antidepressant activity.

Objective: The present study evaluated the antidepressant activities of hydroalcoholic ethanol extract of bulb of Crinum defixum (EECD) using several experimental models

Materials and methods: .Adult Wistar albino rats were subjected to antidepressant activity of different animal models such as Forced swim test, Tail suspension test, Locomotor activity. Biochemical estimation of MAO, estimation of total protein

Results and discussion: The effects of EECD on forced swim test performance were evaluated. The EECD (400 mg/kg, p.o.) showed dose-dependent significant results in all the methods ($p < 0.001$). EECD (400mg/kg) significantly ($p<0.001$) reduced the brain MAO-A levels compared to respected vehicle treated group.

The phytochemical screening revealed the presence of alkaloid, flavonoids, β-sitosterol, and steroids. LCMS study confirms the presence of lycorine which responsible for the antidepressant activity.

Conclusions: The results of the study for the first time show that the plant possesses neuropharmacological activity. Future research should focus on the identification and the antidepressant activity of the constituents from this plant.

Keywords: Crinum difixum, antidepressant, ethanolic extract, lycorine

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INTRODUCTION

C. defixum Ker-Gawl (Amaryllidaceae) is a bulbous herb which has a wide geographical distribution in India and is common on riverbanks and swampy places in Deccan and Bengal. The bulb of this plant is fusiform, stoloniferous base and it has cylindrical neck. Flowers are sessile, fragrant at night and tinged with red. Traditionally the leaves were used to treat pimples, body swelling, dropsy, carbuncles, paronychia, leprosy, fever, diarrhea and leucorrhea. The juice prepared from the leaves of this plant is instilled into the ear to treat otitis. The bulb has nauseant, emetic, emollient and diaphoretic properties.

It is also used in the treatment of burns, whitlow and carbuncle. C. defixum is reported to contain the active constituents such as caranine, crinamine, crinine, galanthamine, galanthine, haemanthamine and hippestrine. Recently a new alkaloid 5α-hydroxyhomolycorine has also been reported. [1] In view of the traditional claims and due to variety of active constituents in the plant, the present study was carried out to evaluate the anti-antidepressant activity of ethanolic extracts

2.1 METHODS

Plant material

The entire Crinum defixum Ker-Gawl were collected in Pakal forest area of Warangal district, Andhra Pradesh in the month of January. The collected plant material was identified and authenticated by RPRC, BBSR (No. 11366) has been kept in the museum for future reference. The bulbs were thoroughly checked for the presence of any foreign organic matter and then the bulbs were coarsely size reduced. Preparation of the extract The coarse powder (100 g) prepared from the bulbs of Crinum defixum was extracted successively with various solvents in increasing order of polarity from petroleum ether, chloroform, ethyl acetate and ethanol.

The filtrates obtained were concentrated under reduced pressure and the extractive values were calculated. The dried extracts were used for preliminary phytochemical screening by performing various chemical tests.

2.1.1. Maximum tolerated dose (MTD):

The maximum tolerated dose was found to be 2000 mg/kg,.p.owhich was determined according to the OECD Guidelines 423. The doses were selected for the antidepressant activity are100, 200 and 400mg/kg.

2.2. ANTIDEPRESSANT ACTIVITY USING ANIMAL MODELS

All the experimental protocol approved by Institutional Animal Ethical Committee (Regd. No. 1053/PO/Re/S/07/CPSCSEA, approval no. 29/IAEC-IPT/17, 38/IAEC-IPT/17).

2.2.1 Forced swim test [1]

Albino rats weighing 150-170 g were grouped to use and brought to the laboratory one day before the experiment and were housed separately in poly propylene cages with free access to food and water. Animals are selected randomly from total 36 of animals and made 6 groups each containing 6 animals. Rats were placed individually in a bucket (40cm Height & 18 cm diameter,Temp^{r}25±1^{0}C) which were filled with water to a 20 cm depth. Two sessions were conducted an initial 15 minute training session (pretest session), The rats were removed 15 min later, dried and placed in their home cage followed by 24 hour later by 6 minute test session. The rats were treated with different doses of extracts (100,200,400mg/kg,p.o), imipramine, fluoxetine and control are given to other 3 groups for 7days once in a day. On 7thday the behavioral response (immobility, climbing, and swimming) was recorded. The results of the treated groups werecompared with that of control. Animals were selected randomly from total 36 of animals and made 6 groups each containing 6 animals.

Group-1: Served as control, received vehicle only (10ml/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-2: Served as stndard, recived Imipramine (15mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-3: Served as, standard received fluoxetine only (20mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-4: Served as test, received*EECD* (100mg /kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-5:Served as test, recived*EECD* (200mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-6: Served as test received *EECD* (400mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.

2.2.2.Tail suspension test [02]

Male mice weighing 20-30 g were grouped to use and brought to the laboratory one day before the experiment and were housed separately in poly propylene cages with free access to food and water. The rats were treated with different doses of

extracts (100, 200, 400 mg/kg, p.o) imipramine fluoxetine and control are given to other 3 groups for 7days once in a day.On 7thday, 60 min after administration, testing was done and duration of immobility was recorded .Here the mice were suspended on the edge of a shelf 58 cm above the table top by adhesive tap placed approximately 1 cm from the tip of the tail.The duration of immobility was recorded for a period of 6 min.
Animals were selected randomly from total 36 of animals and made 6 groups each containing 6 animals.

Group-1: Served as control, recived vehicle only (1ml/100gm) (p.o.) 60 min prior to the induction of depression by Tail suspension test.
Group-2: Received imipramine (15mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test
Group-3: Received fluoxetine (20mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test
Group-4: Received *EECD* (100mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-5: Received *EECD* (200mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.
Group-6: Received *EECD* (400mg/kg) (p.o.) 60 min prior to the induction of depression by forced swim test.

2.2.3. Locomotor activity[03]

Albino mice weighing 25-30 g were grouped into 3 each consisting of 6 animals. Food but not water was withdrawn 12 hours before the start of the experiment. Activity was recorded after 30 minutes of drug administration. The rule out effect of the extract on immobility period ,horizontal locomotor activities of control and test animals were recorded for 10 min using actophotometer. The mice were assessed to show immobility behaviour when placed in actophotometer.
Animals were selected randomly from total 18 of animals and made 3 groups each containing 6 animals. :
Group 1: Served as control,received vehicle only (1ml/100gm) (p.o.) 60 min prior to testing of locomotor activity by photoactometer
Group-2: Received imipramine (15mg/kg) (p.o.) 60 min prior to the testing of locomotor activity by photoactometer
Group-3: Received *EECD* (400 mg/kg) (p.o.) 60 min prior to testing of locomotor activity by photoactometer

2.3 MECHANISM OF ACTION IN TAIL SUSPENSION TEST[03]

Male mice weighing 25-35 g were used preferentially. The animals of respective group were treated with extract or veichle (p.o) for 7 days. On 7th day, 45 minute after treatment, Baclofen Prazosine, sulpiride and clorhexidine were administered (p.o) to group 3, 4, 5, 6, 7, 8, 9, 10 respectively. Here the mice were suspended on the edge of shelf 58cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. Mice were considered immobile when they hang passively and completely motionless for at least 1 min. The duration of immobility was recorded for a period of 6 min.
Animals were selected randomly from total 60 of animals and made 10 groups each containing 6 animals.
Groups-1: Served as control, received vehicle for 7 days (p.o)
Group-2: Serve as test, received *EECD* (400mg/kg) for 7 days.
Group-3: Serve as served as prazosine control, receivedvehicle for 7 days (p.o.) and on 7th day 45 minute after treatment, prazosine 1mg/kg was administered (p.o.).
Group-4: Received *EECD* (400mg/kg) for 7 days (p.o.) and on 7th day 45 minute after treatment, Prazosine 1mg/kg was administered (p.o.).
Group-5: Serve as Baclofen control, received veichlefor 7 days (p.o.) and on 7th day 45 minute after treatment, Baclofen 10mg/kg was administered.
Group-6: Received *EECD* (400mg/kg) f or 7 days (p.o.) and on 7th day 45 minute after treatment, Baclofen 10mg/kg was administered.
Group-7: Serveas Sulpiride control, received veichlefor 7 days (p.o.) and on 7th day 45 minute after treatment, Sulpiride 50mg/kg was administered.
Group-8: Received *EECD* (400mg/kg)f or 7 days(p.o.) and on 7th day 45 minute after treatment , Sulpiride 50 mg/kg was administered.
Group-9: Serve as Chlorohexidine 5mg/kg control, received veichlefor 7 days (p.o.) and on 7th day 45 minute after treatment, ciproheptidine 10mg/kg was administered.
Group-10: Received *EECD* (400mg/kg) for 7 days (p.o.) and on 7th day 45 minute after treatment Chlorhexidine 5mg/kg was administered.

2.4BIOCHEMICAL ESTIMATION OF MAO (A and B) ENZYME[4,5]

Animals exposed to the forced swimed test were sacrificed on the 7th day, after 6 min exposure to the forced swim test and the brain sample were collected immediately in an ice plate. The collected

brain samples were washed with cold 0.25 sucrose .0.1 M ,tris and 0.02M EDTA buffer (pH 7.4) and weighed. The whole procedure of brain isolation was completed within five minutes. Rat brain mitochondrial fractions were prepared following the procedure of Schurr and Livene.Briefly ,the buffer washed brain samples was homogenized in 9 volumes of cold 0.25M sucrose ,0.1M tris ,0.02M EDTA buffer (ph 7.4) and centrifuged twice at 800g for 10 min at 40c in cooling centrifuge ,the pellets were discarded.

The supernatant was then centrifuged at 12000 g for 20 min at 4°c in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-Tries-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4°c for 20 min. The mixture was then centrifuged at 15000 g for 30 min at 0° and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard. The assay mixture contained 100 µl of 4 mM 5-hydroxytryatpamine and 100 µl of 0.1 M benzyl amine as the specific substrate for MAO-A and MAO-B, respectively, 150 µl solution of mitochondrial fraction and 2.75 ml sodium phosphate buffer (100 mM, pH 7.4).For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 0.1 M Benzylamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer.

This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing sodium phosphate buffer and benzylamine.For estimating MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryptamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Systronics 2203, Bangalore, India). This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-hydroxytryptamine.

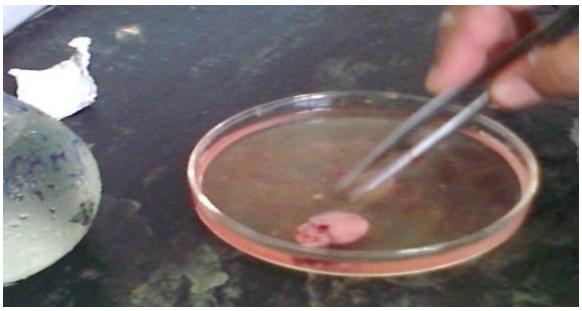

Figure 1: Isolation of rat brain for MAO estimation

The difference in absorbance in 5 minute was calculated according to the method described in "methods of enzymology" by Charles M And mc ewen J (charls M and Mc ewen J,1971).According to this method,one spectrophotometric unit of enzyme is defind as the amount of enzyme which produces an initial rate of change in optical density of 0.001/minute .From this formula the unit of enzyme was calculated and expressed as units present per gm of protein.

2.5 ESTIMATION OF TOTAL PROTEIN [6]

Different dilutions of BSA solutions were prepared by mixing stock BSA solution (5 mg/ml) and water in the test tube. The BSA range is 0.05 to 5 mg/ml.From these different dilutions, 0.2 ml protein solution was pipette out to different test tubes and 2 ml of alkaline copper sulphate reagent (analytical reagent) was added. The solutions were mixed well. This solution was incubated at room temperature for 10 minutes. Then 0.2 ml of reagent Folin ciocalteau solution (reagent solutions) was added to each tube and incubated for 30 min. The absorbance was measured at 600 nm.The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of unknown sample was checked to determine the concentration of the unknown sample using the standard curve plotted above.

Table-1: Standard graph for proteins:

BSA(µg/ml)	Sample Volume(ml)	Alkaline $CuSO_4$(ml)	Lowry Reagent(ml)	Absorbance at 600nm
0.05	0.2	2	0.2	0.088
0.1	0.2	2	0.2	0.133
0.2	0.2	2	0.2	0.258
0.4	0.2	2	0.2	0.353
0.6	0.2	2	0.2	0.372
0.8	0.2	2	0.2	0.453
1.0	0.2	2	0.2	0.560
3.0	0.2	2	0.2	2.644
5.0	0.2	2	0.2	4.754

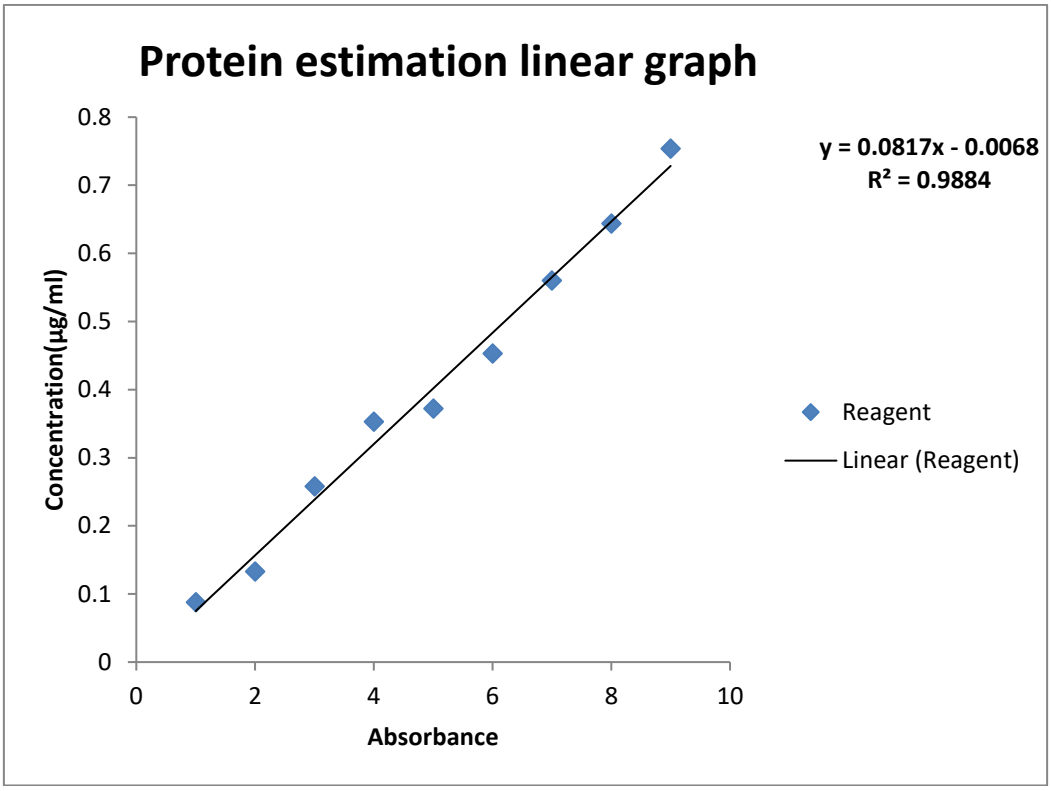

Figure 2: Estimation of total protein by Lowery method.

LCMS

Sample Preparation

The dried and powdered aerial parts and bulbs of the plant (200 mg) were macerated with 5 mL of 2% hydrochloric acid for 5 h in an ultrasonic bath at 40 °C, the extract was made alkaline with 1 mL of 26 % ammonium hydroxide and the volume was adjusted to 10 mL in a volumetric flask with distilled water. The extract was dissolved in 1 mL 0.1% TFA, passed through a 0.45-µm filter (Grace Davison, USA), and 20 µL of the filtrate was injected into the HPLC column for analysis.

Chromatography

The analysis of the samples and validation experiments were carried out using a liquid chromatografic system (Shimadzu LCMS) a thermostatted column compartment, a manual injector with 20 µL loop (Rheodyne 7725i), a diode array detector (DAD). The chromatographic resolution was performed with an isocratic mobile phase including methanol 70% on a Hichrom C18 column (5 µm particle size, 250 mm, 4.6 mm) at a flow rate of 1 mL/min and λmax 290 nm at 25 oC. The injection volume was 20 µL. The chromatographic run time was 45 min.

3. RESULT

3.1 FORCED SWIM TEST (IMMOBILITY)

Acute dose treatment of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with decrease the immobility period in forced swim test (FST) as compared to control group indicating significant antidepressant like effect of the extract. Among these doses (100 and 400 mg/kg,p.o) of *EECD* shows a decreased immobility periods with significance ($p<0.001$), and (200 mg/kg) of *EECD* shows a decreased immobility periods with significance ($p<0.05$).

Table: 2 Effect of *EECD* on immobility period in FST after single oral dose administration.

GROUPS	Treatment (p.o)	Immobility period (sec) Mean ± SEM
I	Vehicle treated 10ml/kg (p.o)	104 ± 2.9
II	Imipramine 15mg/kg(p.o)	55± 3.5**
III	Fluoxetine 20mg/kg(p.o)	39 ± 3.1**
IV	*EECD* 100mg/kg(p.o)	50 ± 6.5**
V	*EECD* 200mg/kg(p.o)	71.5 ± 13.7*
VI	*EECD* 400mg/kg(p.o)	18.83 ± 8.8**

EECD = Ethanolic extract of *Crinum defixum*. Values are mean ± SEM, (n=6) in each group **P<0.01, *p<0.05 as compared to vehicle treated group.

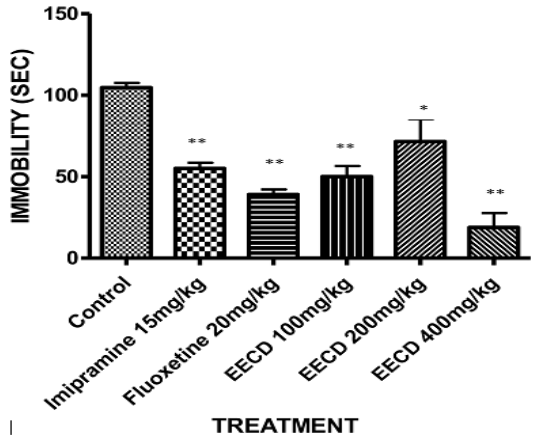

Figure3: Effect of *EECD* on Immobility period in FST after acute oral administration.

Chronic treatment (7 days) of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with decrease the immobility period in forced swim test (FST) as compared to control group indicating significant antidepressant like effect of the extract. Among these doses (100, 200 and 400 mg/kg, p.o) of *EECD* shows a decrease in immobility periods with significance (p<0.01).

Table: 3 Effect of *EECD* on immobility period in FST after chronic dose (7 days) oral administration.

Groups	Treatment(p.o)	Immobility period (sec) Mean ± SEM
I	Vehicle treated10ml/kg	60.1±1.6
II	Imipramine 15mg/kg	25±2.3**
III	Fluoxetine 20mg/kg	9±1**
IV	*EECD* 100mg/kg	22±3.7**
V	*EECD* 200mg/kg	26.3±5**
VI	*EECD* 400mg/kg	7±1.7**

EECD=Ethanolic extract of *Crinum defixum*. Values are mean ± SEM, (n=6) in each group p<0.01 as compared to vehicle treated group.

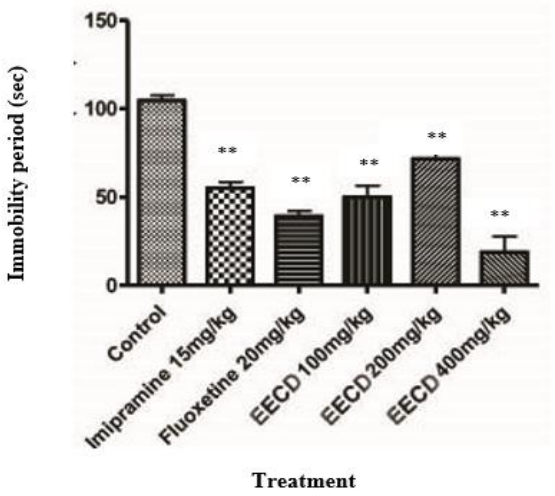

Figure4: Effect of *EECD* on Immobility period in FST after chronic oral (7 days) administration.

3.2FORCED SWIM TEST (SWIMMING)

Acute dose treatment of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with decrease the immobility period in forced swim test (FST) as compared to control group indicating significant antidepressant Among these doses (400 mg/kg, p.o) of *EECD* shows a increase in swimming period with significance (p<0.01), (100 mg/kg) of *EECD* shows a increase the swimming period with significance (p<0.05) and (200 mg/kg) of *EECD* do not show any significance of swimming.

Table: 4 Effect of *EECD* on swimming in FST after acute dose of oral administration.

Groups	Treatment (p.o)	(Swimming period) Mean ± SEM
I	Vehicle treated 10ml/kg	135.3±4.2
II	Imipramine 15mg/kg	185±3.6**
III	Fluoxetine 20mg/kg	201±3.2**
IV	*EECD* 100mg/kg	190±6.5*
V	*EECD* 200mg/kg	166±15.1
VI	*EECD* 400mg/kg	204.8±13.9**

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ± SEM, n=6 in each group, **p<0.01, *P<0.05 as compared with vehicle treated group.

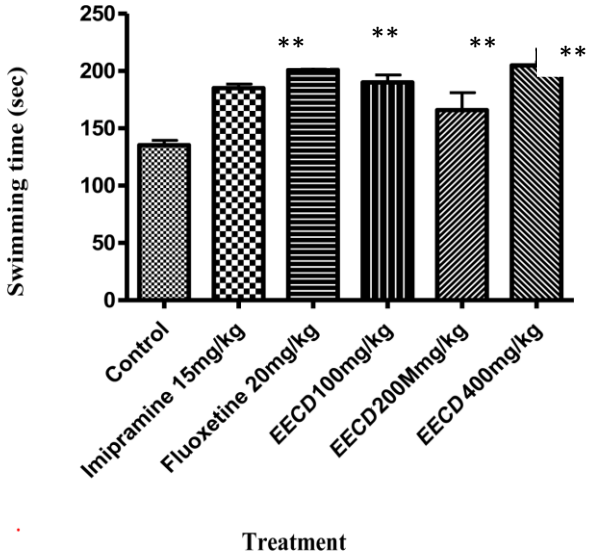

Figure 5: Effect of *EECD* on swimming time in FST after acute oral administration

Chronic treatment (7 days) of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with in forced swim test (FST) as compared to control group indicating significant antidepressant like effect of the extract. Among these doses (100, 200 and 400 mg/kg, p.o) of *EECD* shows a decreased in immobility periods with significance (p<0.01).

Table: 5 Effect of *EECD* on swimming time in FST after chronic dose (7 days) oral administration.

GROUPS	Treatment (p.o)	Swimming time Mean ± SEM
I	Vehicle treated10 ml/kg	179.5±5.5
II	Imipramine 15 mg/kg	215±2.5**
III	Fluoxetine 20 mg/kg	231±1.843**
IV	*EECD* 100 mg/kg	218±3.7**
V	*EECD* 200 mg/kg	213.3±6.6**
VI	*EECD* 400 mg/kg	231±1.8**

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ± SEM, n=6 in each group. *p<0.01 as compared with vehicle treated group

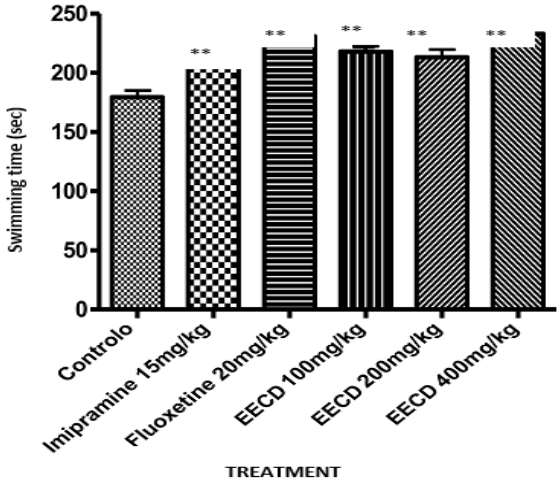

Figure 6: Effect of *EECD* on swimming period in FST after chronic dose (7 days) oral administration.

3.3FORCED SWIM TEST (CLIMBING)

Acute dose treatment of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with increase the numbers of climbing in forced swim test (FST) as compared to control group indicating significant antidepressant like effect of the extract. Among these doses (400 mg/kg,p.o) of *EECD* shows a decreased immobility periods with significance (p<0.001), (100 mg/kg) of *EECD* shows a decreased immobility periods with significance (p<0.05),and (200 mg/kg) of of *EECD* do not show any significance of climbing.

Table: 6 Effect of *EECD* on climbing in FST after acute dose of oral administration

GROUPS	Treatment (p.o)	No of Climbing ,Mean±SEM
I	Vehicle treated10 ml/kg	0
II	Imipramine 15 mg/kg	9.6±2.9**
III	Fluoxetine 20 mg/kg	6±0.6*
IV	*EECD* 100 mg/kg	6.3±0.8*
V	*EECD* 200 mg/kg	2.1±0.7
VI	*EECD* 400 mg/kg	10.3±1.4**

*EECD= ** olic* extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group .p<0.01, P<0.05 as compared with vehicle treated group.

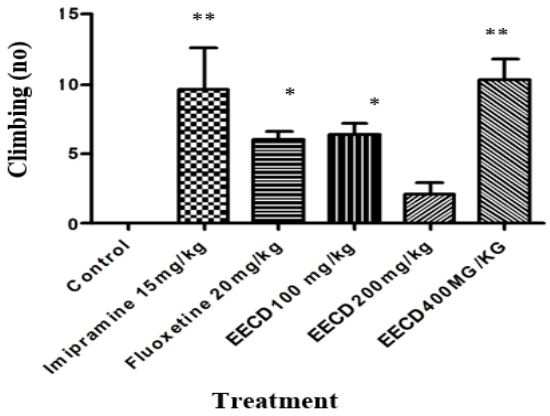

Figure 7: Effect of *EECD* on no of Climbing in FST after acute oral administration

Chronic treatment (7 days) of *EECD* (100, 200 and 400 mg/kg, p.o) to rats with decrease the immobility period in forced swim test (FST) as compared to control group indicating significant antidepressant like effect of the extract. Among these doses (100, 200 and 400 mg/kg,p.o) of *EECD* shows a decreased in immobility periods with significance (p<0.01).

Table: 7 Effect of *EECD* on climbing in FST after chronic dose of oral administration.

GROUPS	Treatment (p.o)	Climbing ,Mean ± SEM
I	Vehicle treated10 ml/kg	2.666±0.421
II	Imipramine 15 mg/kg	61.5±6.941**
III	Fluoxetine 20 mg/kg	4.333±1.021
IV	*EECD* 100 mg/kg	6.1666±1.701
V	*EECD* 200 mg/kg	7.333±0.988
VI	*EECD* 400 mg/kg	2.333±1.173

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, n=6 in each group. p<0.01 as compared with vehicle treated group.

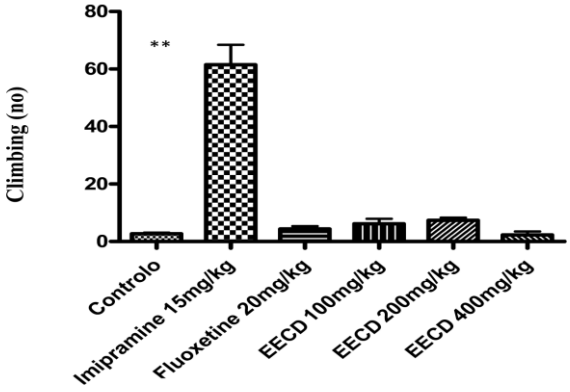

Figure 8: Effect of *EECD* on no of Climbing in FST after chronic (7 days) oral administration in rat.

3.4 TAIL SUSPENSSION TEST

EECD (100,200 and 400 mg/kg, p.o) administered to rats for 7 successive days with decreased Immobility periods significantly in a dose dependant manner as compared to control in TST.A dose of (400 mg/kg p.o.) of the extract showed the most potent antidepressant activity.

Table: 8 Effect of multi dose of *EECD* (in different) doses, *Imipramine* and Fluoxetine on forced swim test in rat.

GROUPS	Treatment (p.o)	Immobility period (sec) Mean ± SEM
I	Vehicle treated10ml/kg	142.5±3.818
II	Imipramine 15mg/kg	110±12.110*
III	Fluoxetine 20mg/kg	93.833±9.833**
IV	*EECD* 100mg/kg	105±4.830
V	*EECD* 200mg/kg	128.667±4.409*
VI	*EECD* 400mg/kg	105±4.830**

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM (,n=6)in each group.* p<0.05,**p<0.01 as compared with vehicle treated group.

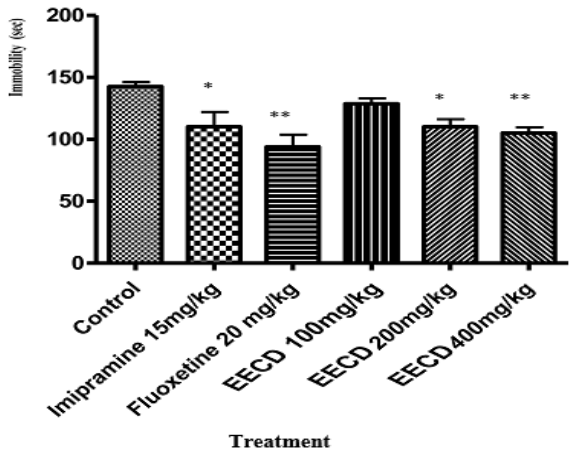

Figure09: Effect of EECD on Immobility period in TST after chronic (7 days) oral administration in rats

3.5 LOCOMOTOR ACTIVITY

In FST & TST study, the*EECD* were administered orally for 7 successive days and among these the *Ethanolic* extract (400 mg/kg) produced a significant antidepressant effect in FST & in TST.So this extract was selected for investigating the locomotors activity in rats ,Biochemical estimation of monoamine oxidase A and B in rats possible mechanism for its antidepressant effect in mice.

Chronic treatment of *EECD* (400mg/kg,p.o) administered for 7 successive days to rats did not show any significant change in the locomotors function as compared to the vehicle treated group.

Table: 9 Effect of *EECD*, Imipramine on forced swim test in rat.

Groups	Treatment (p.o)	Mean ± SEM
I	Vehicle treated10ml/kg	239±27.004
II	Imipramine(15mg/kg)	351.333±58.188
III	Extract (400 mg/kg)	298.833±32.315

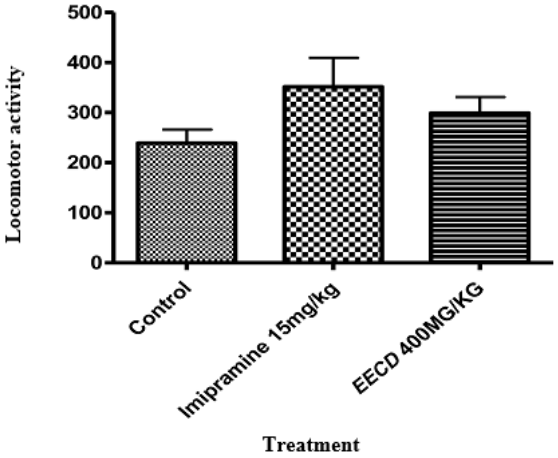

Figure 10: Effect of *EECD* on locomotor activity in rats

3.6 Mechanism of action study

In FST & TST study, the*EECD* were administered orally for 7 successive days and among these the *Ethanolic* extract (400 mg/kg) produced a significant antidepressant effect in FST & in TST. So this extract was selected for investigating the possible mechanism for its antide pressant effect in mice. Baclofen (10 mg/kg I.p), Prazosin (1mg/kg I.p) sulpiride and ciproheptidine were administered and prazosin sulpiride and ciproheptidiene were significantly **p<0.01 increase the immobility period as compared to the vehicle treated group. The animals with prazosin sulpiride and cyprohep tidine significantly reversed the immobility time by *EECD* at the dose of 400mg/kg,p.o.Baclofen did not show any significant effect in both the cases.

Table 10: Effect of combination of *EECD* (400MG) with Baclofen, Prazosine sulpiride and Chlorhexidine on immobility period on mice in TST.

Groups	Treatment (p.o/I.p)	**Immobility period (Mean ± SEM)**
I	Vehicle treated10ml/kg	142.5±3.818
II	Extract 400 mg/kg	110±12.110##
III	Control + baclofen	171.5±8.774
IV	Extract +baclofen	102±6.597
V	Control +prazosine	196±8.903**
VI	Extract +prazosine	172±3.473##
VII	Control +sulpiride	242.5±12.7639**
VIII	Extract +sulpiride	233.333±7.264##
IX	Control +chlorohexidine	194±8.925**
X	Extract +chlorohexidine	137±12.412##

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM ,n=6 in each group.

**p<0.01 as compared with vehicle treated group and## p<0.01 as compared to EECD(400 mg/kg)

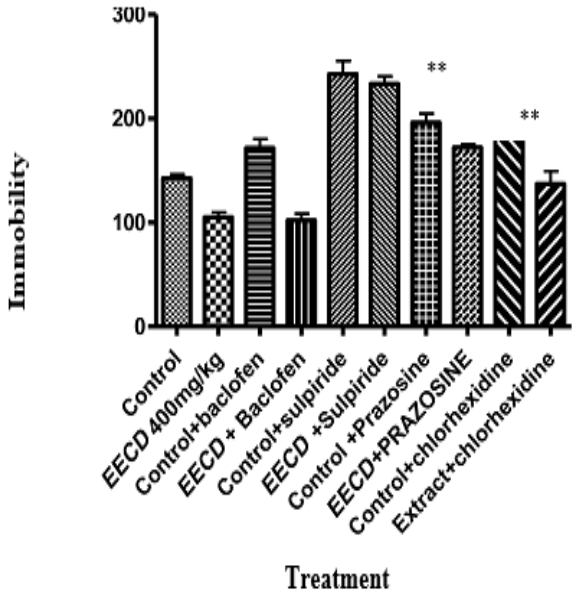

Figure 11:*EECD= Ethanolic* extract of *Crinum defixum*. Values are Mean ± SEM, n=6 in each group. Prazosin , sulpiride and ciproheptidine increases the Immobility with significance *

*p<0.01 as compared with vehicle treated group. Prazosin , sulpiride and ciproheptidine increases the Immobility with significance ## p<0.01 as compared with *EECD* (400 mg/kg) .

3.7 BIOCHEMICAL ESTIMATION OF MAO:

Monoamine oxydase-A(MAO-A)

Chronic treatment of *EECD* (400mg/kg) was administered for 7 consecutive days to rat significantly (p<0.01) reduced the brain MAO-A levels compared to respected vehicle treated group.

Table: 11 Effect of *EECD* on MAO-A in rat.

Group	Treatment (p.o)	Mean ± SEM
I	Vehicle treated10ml/kg	7.74±1.081
II	Imipramine 15mg/kg	1.58±0.487**
III	Extract (400 mg/kg)	2.577±0.533*

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group.

**p<0.01 as compared with veichle treated group.

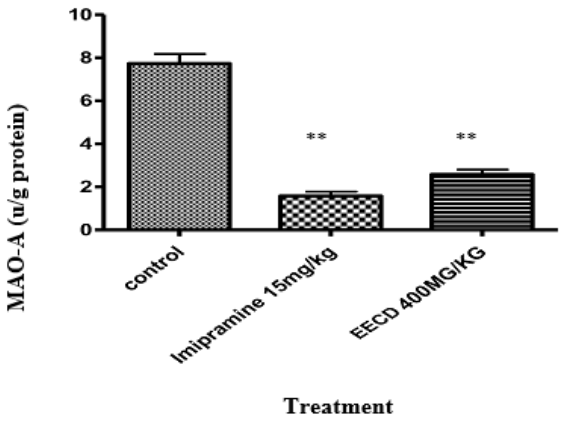

Figure 12: Effect of *EECD* on MAO-A activity in rat.

Monoamine MAO-

Chronic treatment of *EECD* (400mg/kg) was administered for 7 consecutive days to rat significantly (p<0.001) reduced the brain MAO-A levels compared to respected vehicle treated group.

Table: 12 Effect of *EECD* on MAO-B in rat.

Groups	Treatment (p.o)	Mean ±SEM
I	Vehicle treated10ml/kg	45±0.288
II	Imipramine 15mg/kg	7.006±0.597
III	Extract (400 mg/kg)	6.942±0.592

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group.p<0.05 as compared with veichle treated group.

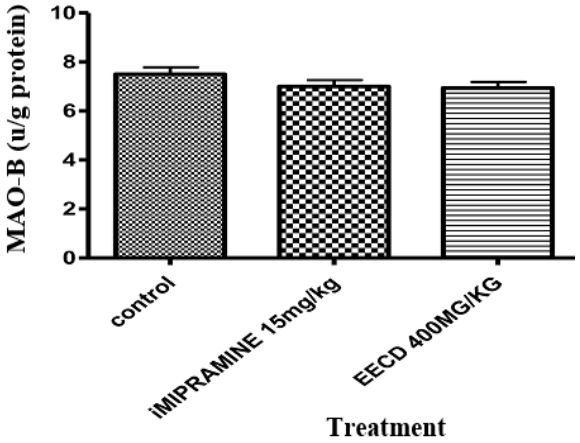

Figure 13: Effect of *EECD* on MAO-B activity in rat.

LCMS

The analysis of the samples and validation experiments were carried out using a liquid chromatografic system. The chromatographic run time was 45 min. All the calculations regarding the quantitative determinations were carried out by an external standard method based on peak areas. The chromatograms of the standard lycorine and plant

extracts are shown in Figures 14 and 15. The identification of lycorine in plant specimens was achieved by comparison of the retention time and peak area with those of standard lycorine. The Rt of the extract was found to be 1.63 and standard was found to be 1.55 respectively.

The method was validated in accordance with linearity, precision, recovery and limits of detection and quantification. This simple, rapid and reliable HPLC method is suitable for the quantitative analysis of lycorine, which is a biologically important Amaryllidaceae alkaloid.

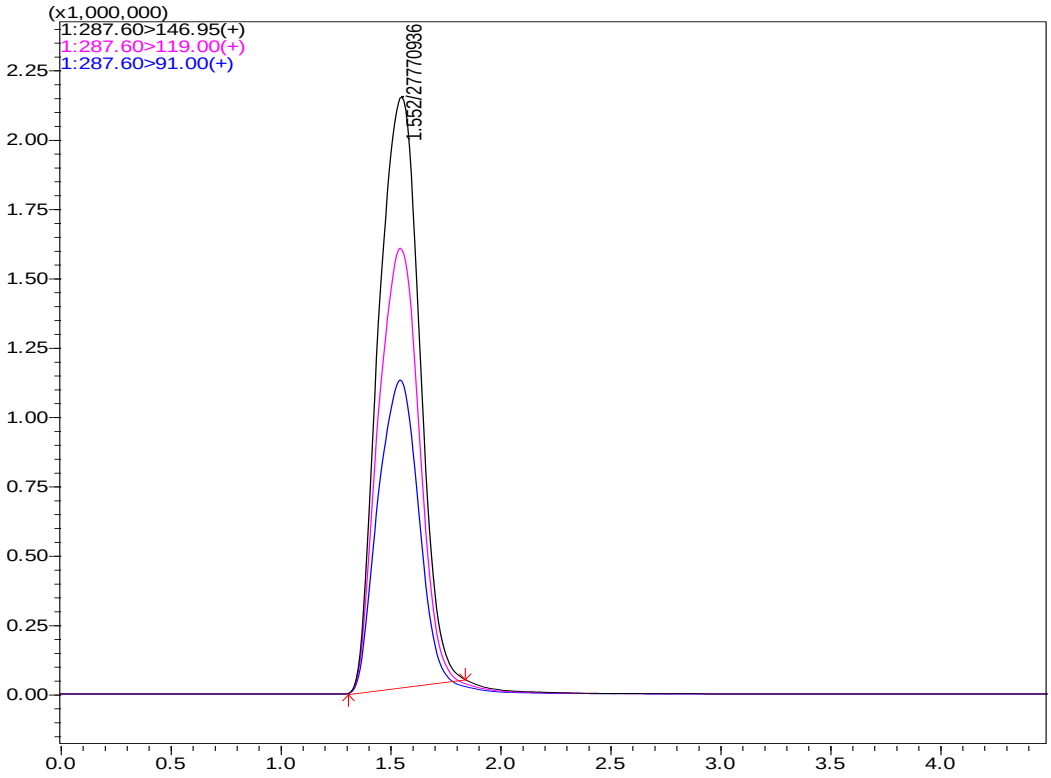

Fig. 14 LCMS chromatogram of standard lycorine

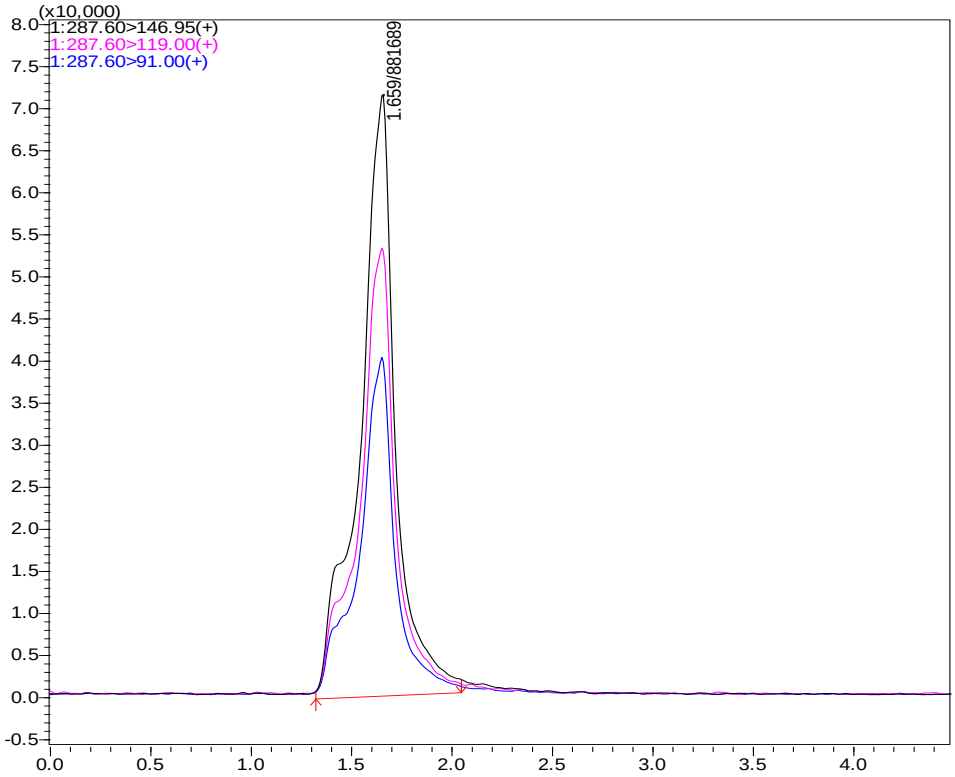

Fig.15 HPLC chromatogram of standard lycorine C. difixum

4 DISCUSSION

In the present investigation, experimental antidepressant activity was measured using the *Ethanolic* extract of *Crinum defixum* in rodents. Based on the chemical structures and traditional uses different herbal extracts were implicated for treatment of many diseases. *Crinum defixum* is traditionally used for anti-depressant, anti-epileptic, wound healing activity etc.

Depressive disorder is the most frequent psychiatric condition now a day. In the fields of psychology and psychiatry, the terms depression states that a depressed mood is often reported as feeling depressed, sad, helpless, and hopeless. In traditional colloquy, "depressed" is often synonymous with "sad," but both clinical and non-clinical depression can also refer to a conglomeration of more than one feeling. It is the most leading cause of death and 20% of the adult population suffers from this condition . The main cause of depression is genetic predisposition and neurological changes in brain.

During depression there is an increase in monoamine oxidase enzyme system (MAO-A & MAO-B) and decrease in the concentration of monoamine levels, mainly serotonin dopamine &5-hydroxy tryptamine.

The immobility in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despite, which in turn may reflect depressive disorder in humans. It is suggested that mice or rat forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despire which can be reduced by several agents which are therapeutically effective in human depression. Many hypothesishave been advanced to explain the physical adaptation for depression i.e. the immobility response observed in the forced swim (FST)test and tail suspension test (TST) respectively. In the FST, Tricyclic antidepressants that selectively decrease in immobility increase climbing without affecting swimming.Serotonin reuptake inhibitors also reduce immobility but increase swimming instead of climbing.

The present study was used to investigate whether the *leaves* of the plant *C. defixum*produce behavioural modification in rats and mice.The antidepressant effects were assessed in the forced swim test (FST) and tail suspenssion test (TST). In addition to this the animals were treated with extract in combination with different antagonists involved in monoaminergic transmission and also with GABA agonist. We analyze the rat brain for monoamine oxidase A and B (MAO-A and B) activities ex-vivo after extract treatment.

Antidepressant activity of *C. defixum* was evaluated in rats employing FST,and in mice employing TST . These models are most commonly used models for depression activity these models are widely employed for rodents to predict antidepressant potential by decrease of immobility period produsedby several different classes of antidepressant drugs. In the study,the ethanolic extracts (100,200 and 400mg/kg) were administered for 7 successive days and among these the 400mg/kg dose produced a significant antidepressant effect in TST and FST. This dose showed the most potent result with highest decrease in immobility in FST and TST and increase in swimming time and no action in case of climbing in FST. Thus this extract was selected to investigate the possible mechanism of antidepressant like activity.

Observing the forced swim test of *C. defixum*, these extract may affect the climbing behaviour and no selective action was observed. Therefore the extract is no selective effect in trycyclic antidepressant but the serotonergic pathway may be responsible for the antidepressant effect.

The extract at the dose of 400mg/kg p.o. did not show any significant change in locomotor functions in rats as compared to control, so it did not produce any motor effects. This conforms the assumptionthat the antidepressant effect of the extract is specific and not false positive. The effect of the ethanolic extract at 400mg/kg was significantly reversed by pre-treatment of the animals with prazosine (α1 adrenoceptor antagonist) sulpiride (serotonin receptor antagonist) and chlorhexidine (dopamine antagonist). This suggests that the extract might produced an antidepressant like effect by interaction with dopaminergic, adrenergic and serotonergic pathway.Hence increasing the levels of Dopamine serotonin and Norepinephrine and decrease in the levels of GABA in rodent brain.

Levels of monoamines like Norepinephrine and serotonin are decrease in depression, so drugs like tricyclic antidepressants and monoamine oxidase inhibitors, which enhance the levels of these monoamines, have been used as antidepressant drugs. There are two GABA hypothesis of antidepressant action: an increase in GABA-A neurotransmeter or a decrease in GABA-B neurotransmeter may contribute to this action. Thus GABA,Receptor antagonism may serve as a basis for the generation of novel antidepressant.

The *Ethanolic* extract of *C. defixum* reduced the rat whole brain MAO-A activities as compared to control, indicating that this extract inhibited the metabolism of monoamines, particularly serotonin and nor adrenaline. MAO regulates the metabolic

degredation of catecholamines;inhibition of this enzyme causes a reduction in metabolism and subsequent decrease in the concentration of biogenic amines. The *Ethanolic* extract of *C. defixum* does not reduce the MAO-B activities, Though MAO-B is responsible for dopamine metabolism, it concludes that *C. defixum*is not acting through Dopaminergic pathway,while acting through serotonin and Norepinephrine. So the extract of *C. defixum* showed the antidepressant activity probably by inhibiting MAO activity.

5 CONCLUSION

Although there are many effective anti-depressant drugs available for therapeutic care, agents which can improve the monoamine levels or inhibition of monoamine oxidase enzyme system. can be an add-on therapy to the anti-depression agents. The ethanolic extract of *Crinum defixum* significantly decreases the immobility period while increase in swimming time and no effect of climbing in FST and TST. It conforms that the assumption that the antidepressant effect of the extract was specific and not false positive. The most potent dose among all the doses was 400mg/kg dose. The extract showed antidepressant activity probably by inhibiting MAO-A and MAO-B while increase in monoamine levels like noradrenaline (NA), serotonin(5-HT) and dopamine (DA). According to the literature and phytochemical screening.

Further the antidepressant action may be due to presence of Lycorine, confirmed by the LCMS analysis and the compounds have been reported to have this activity. Therefore the ethanolic extract of *Crinum defixum* may have potential therapeutic value for the management of depressive disorders.

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