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STUDY OF NEUROPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *OXALIS CORNICULATA LINN* ON ANIMAL MODELS OF DEPRESSION

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ABSTRACT

Depression and anxiety disorders are the most common mental illnesses, each affecting in excess of 10-15% of the population at some time in their lives. Approximately 450 million people suffer from a mental or behavioural disorder according to the WHO report, resulting in 12.3% of the global burden of disease. Though various therapeutic regimens are used to treat anxiety and depressive disorders; but due to their side effects and dependence liability their use in clinical practice is limited. Research studies on different medicinal plants in various animal models has widely contributed in the search of new therapeutic products for the treatment of various neurological disorders. Hence the present study was undertaken to evaluate the neuroprotective activity of Methanolic extract of *Oxalis corniculata Linn* on animal models of depression.

I. INTRODUCTION

Depression and anxiety disorders are the most common mental illnesses, each affecting in excess of 10-15% of the population at some time in their lives. Depressive episodes are characterized by depressed or sad mood, pessimistic worry, diminished interest in normal activities, mental slowing and poor concentration, insomnia or increased sleep, significant weight loss or gain due to altered eating and activity patterns, psychomotor agitation or retardation, feelings of guilt and worthlessness, decreased energy and libido, and suicidal ideation, occurring most days for a period of at least 2 weeks. Depressive symptoms also can occur secondary to other illnesses such as hypothyroidism, Parkinson's disease, and inflammatory conditions[1]. Depressive disorders are characterized by the impairment of mood regulation. Many researchers have suggested that by 2020, depression will become the second leading cause of disease worldwide. Depression also complicates the course and outcome of other illnesses among older adults. Depressive disorders are also strong predictors of suicide for older adults, suggesting that significant depressive symptoms may indicate a serious threat to the health & survival of older adults[2]. Though various therapeutic regimens are used to treat anxiety and depressive disorders; but due to their side effects and dependence liability their use in clinical practice is limited. Research studies on different medicinal plants in various animal models has widely contributed in the search of new therapeutic products for the treatment of various neurological disorders[3].

Depression, is considered as an affective disorder characterized by the symptoms of depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self- worth, disturbed sleep or appetite and also poor concentration. It often comes with symptoms of anxiety and can lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. Depression at its worst can lead to suicide. It affects people in all communities across the world and is a significant contributor to the global burden of disease[4]. Depression occurs frequently in patients with neurologic disorders, particularly cerebrovascular disorders, Parkinson's disease, dementia, multiple sclerosis and traumatic brain injury. Depression is approximately twice as common in women as in men, and the incidence increases with age in both sexes.[5]

There has been a marked shift in the last decade in our understanding of the pathophysiology of major depression. In addition to the older idea that a deficit in function or amount of monoamines (the monoamine hypothesis) is central to the biology of depression, there is evidence that neurotrophic and endocrine factors play a major role[6].

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Neuroprotection is the concept of providing a treatment that prolongs the brain's tolerance to ischemia[7]. It is an effect that may result in salvage, recovery, or regeneration of the nervous system, its cells, structure and function. There are many neurochemical modulators of the nervous system damage[8]. There is progressive deterioration of neurobiological tissue during neurodegeneration. Therefore any therapeutic strategy that may be either an intervention, a drug or a treatment and is used to prevent the neurons from dying may be termed as neuroprotection[9].

From time immemorial, the use of plants as a basic remedy for simple & uncomplicated diseases have been practiced due to their easy availability, cost effectiveness, time tested & less hazardous side effects[10] .

Previous studies on the phytochemical analysis of Methanolic extract of Oxalis Corniculata linn(MEOC) suggested the presence of phytoconstituents like alkaloids, carbohydrates, glycosides, physterols, fixed oils and fats, saponins, phenolic compounds and tannins, proteins, amino acids and flavanoids[11]. Phenolic substances and flavonoids are linked to the antioxidant activity and play a vital role in stabilizing lipid peroxidation, by adsorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxides[12].The presence of flavonoids and phenolic compounds in *Oxalis Corniculata Linn* suggests that this plant possesses antioxidant properties and can have neuroprotective propensity[13]. It is well known that reactive oxygen species such as superoxide, hydroxyl and peroxyl radicals are generated in a condition of oxidative stress that play a vital role in the degenerative and the pathological processes of various diseases like ageing, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disease etc [14] . Despite of the severity and high prevalence of these diseases, the allopathic system is yet to provide a satisfactory antidote[15]. Keeping in view of the above ideas, the present study has been undertaken to study the beneficial effects of methanolic extract of *Oxalis corniculata Linn(MEOC)* on neuroprotection in albino mice with depression.

Oxalis corniculata Linn is commonly known as creeping woodsorrel belonging to the family Oxalidaceae and genus Oxalis. It is commonly known as Indian sorrel in English, Tinpatiya in Hindi and ***saru Tengesi*** in Assamese. It is a well known plant in India with a wide range of biological activity. It is used traditionally as anti-inflammatory, digestive, diuretic, antibacterial, antiseptic, in cardiopathy, hepatopathy, dysentery, diarrhea and skin diseases. It is also used in dyspepsia, wound healing, cancer, piles, dementia and convulsions. It was also reported that Oxalis corniculata linn have hypoglycemic, antihypertensive, antipsychotic, nervous system stimulant & have chronotropic and inotropic effect[16].

The study of the review of literature indicated that *Oxalis Coniculata linn* has been used traditionally in the treatment of dementia and the presence of flavanoids may have neuroprotective propensity[17]. Hence the present study was undertaken to study the neuroprotective activity of MEOC. Imipramine was taken as the standard drug to compare the effects.

II. MATERIALS AND METHOD

The study was conducted in the department of Pharmacology Assam Medical College and Hospital, Dibrugarh after taking due approval from the Instituitional Animal Ethics Committee.

Drugs and Chemicals Used in the Study:

1) The drug imipramine used in our study was obtained from Shine pharmaceuticals, Baroda Gujarat.
2) Vehicle : Normal saline (0.9% NaCl)
3) Methanolic extract of *Oxalis corniculata Linn* (MEOC): Methanol was obtained from HiMedia laboratories Private Limited, Dombivli (Maharashtra), India.
4) Distilled Water

Instruments Used In The Study:

- *Oxalis corniculata Linn*
- Methanol
- Soxhlet apparatus
- Electrical grinder
- Vacuum desiccators
- Glass petridishes
- Flask
- Air tight containers

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- Glass jar
- Plastic string
- Stop watch
- Beakers
- Syringes
- Feeding needles

III. EXPERIMENTAL ANIMALS USED IN THE STUDY

The study was carried out in healthy adult albino mice (Mus musculus) of either sex weighing 20-30 grams. The total number of animals used in our study was ninety. The animals were procured from Central Animal House, Assam Medical College. The animals were housed in standard cages under normal temperature and were maintained on balanced diet (consisting of Bengal gram, wheat, maize and powdered soya bean in sufficient quantity) and water was provided ad libitum during the entire period of the experiment.The animals were housed in standard conditions with natural light and dark cycles. The study was duly permitted by the Institutional Animal Ethics Committee (IAEC) of Assam Medical College, Dibrugarh, Assam-786002 (Regd no 634/02/a/CPCSEA dated 19.05.2002) vide approval number (IAEC/AMC/02 dated 23/11/15). The study was conducted keeping in view with the CPCSEA (Committee for The Purpose of Control and Supervision of Experiments on Animals) guidelines.

The animals were allowed to acclimatize to the laboratory environment for 2 weeks and were provided water and food ad libitum.

Collection Of The Plant Material

The plant *Oxalis corniculata Linn* was collected from in and around the Assam Medical College and Hospital campus.

IV. METHOD OF EXTRACTION OF THE METHANOLIC EXTRACT OF THE WHOLE PLANT OF *OXALIS CORNICULATA LINN*[18]

The extract is prepared by using the Soxhlet apparatus and distillation apparatus .The whole plant of *Oxalis corniculata linn* is collected and dried in a drier table at room temperature. The dried plants are then grounded into powder in an electrical grinder. The finely grounded powder approximately 1450gm was extracted in 500ml of 99.8% methanol and then placed in a porous bag or “thimble” made of strong filter paper, which is then placed in a chamber of the Soxhlet apparatus for 16 hrs. The crude extract obtained was filtered through the whatman paper and the filtrate was evaporated. The extract was collected in glass petridishes, further dried in a vacuum desiccator and finally stored in air tight glass containers. In this way the procedure is repeated several times to yield 262 gm of methanolic extract of *Oxalis corniculata linn (MEOC)* which were further preserved in a sterile glass container at 4˚ until further use. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent.

Acute oral toxicity test:

Acute oral toxicity tests for the Methanolic extract of the whole plant of *Oxalis corniculata Linn* was carried out as per OECD Guidelines 425[19]. The limit test at 2000 mg/kg which required a total of 5 albino mice was used. The mice were fasted overnight prior to the experiment and their body weights were measured.A single dose of MEOC (2000 mg/kg body weight) was dissolved as 1ml/100 gm of body weight in Normal Saline and administered orally to the first animal with the help of a feeding tube. Food was withheld for further 3-4 hours. Based on its mortality or appearance of toxic signs and symptoms, the other four animals were dosed sequentially. The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention during the first 4 hours), and daily, thereafter, for a period of 14 days. Observations were done daily for changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system changes (ptosis,drowsiness, tremors and convulsions). Body weights were determined weekly (Organization for Economic Cooperation and Development, 2008).

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No sign of toxicity and mortality was recorded among the mice at the dose of 2000mg/kg (for the extract); hence arbitrarily 100mg/kg and 200mg/kg was selected for the study. A total of two doses was taken to see the dose dependent effect.

Phytochemical analysis:
Qualitative phytochemical analysis was carried out for MEOC as per the standard methods described by Prashant Tiwari *et al*[20]

Experimental Procedures:

A. Forced swimming test(FST)

Principle: FST is the standard proposed model to test the " behavioral despair" for antidepressant activity. A characteristic behaviour of immobility was induced by allowing the mice or rats to forcefully swim in a restricted space from which they cannot escape. This characteristic behaviour reflects a despair state that can be reduced by drugs or agents which is used therapeutically to treat depression in human beings[21].

Procedure[22]
The experiment will be carried out as per the method followed by Madhavi Eerike and Uma Maheswari. N i.e. the method described by Porsolt et al. (1977). The forced swimming test (FST) is based on the observation that animals develop an immobile posture in an inescapable cylinder filled with water. Depression will be produced by forcing the animals to swim individually in a glass jar containing fresh water of 15 cm height. This will constitute the pretest session. 24 hr later each animal will be forced to swim again. After an initial 2min period of vigorous activity, each animal will assume a typical immobile posture. The total duration of immobility will be recorded in 4min after an initial 2 min period of vigorous activity in a total 6 min test. The total duration of immobility and the change in the immobility period will be calculated after administering the drugs to the different groups in a total 6min test. Drugs will be administered orally 60 min prior to the acute study. In chronic study, drugs will be given orally once daily for 10 days and the last dose will be given 60 min before the experiment.

For all the test groups, MEOC was administered at doses of 100mg/kg and 200mg/kg per orally. Imipramine (standard drug) was given 10mg/kg per orally.

Grouping and treatment schedule f0r forced swimming test:
20 Albino mice of either sex will be divided into 4 groups of 5 mice each. They will be treated as follows:

Group	Number of animals	Treatment
Group A (control)	5	10 ml/kg Normal saline
Group B(test drug 1)	5	MEOC 100 mg/kg
Group C (test drug 2)	5	MEOC 200 mg/kg
Group D (standard drug)	5	Imipramine 10 mg/kg

B. Tail suspension test (tst)

Principle: TST is an important means of evaluating potential antidepressants. When mice are subjected to an unavoidable and inescapable stress, they exhibit a typical immobile posture that has been hypothesized to reflect "behavioural despair" which in turn reflects the depressive disorders in human beings. Known antidepressants reduce the immobility that mice display after the unsuccessful attempts to escape when suspended by the tail[21].

Procedure[22]:
The experiment will be carried out as per the method followed by Madhavi Eerike and Uma Maheswari. N i.e the method described by Steru et al.(1985). The animals will be hung by the tail on a plastic string 75cm above the surface with the help of an adhesive tape. The duration of immobility will be recorded during the last 6min of the observation period of a total 8min test. Mice will be considered immobile only when they hung passively and completely motionless. Drugs will be administered orally 60 min prior to the acute study. In chronic study, the drugs will be given orally once daily for 10 days and the last dose will be given 60 min before the experiment. Changes in the immobility duration will be studied after administering drugs to the different groups of animals.

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For all the test groups, MEOC was administered at doses of 100mg/kg and 200mg/kg per orally. Imipramine (standard drug) was given 10mg/kg per orally.

Grouping and treatment schedule of tail suspension test:
20 albino mice of either sex will be divided into 4 groups of 5 mice each. They will be treated as follows:

Group	Number of animals	Treatment
Group A (control)	5	10 ml/kg Normal saline
Group B(test drug 1)	5	MEOC 100 mg/kg
Group C (test drug 2)	5	MEOC 200 mg/kg
Group D (standard drug)	5	Imipramine 10 mg/kg

V. STATISTICAL ANALYSIS

The results were expressed as mean± S.E.M. The data were statistically analysed by using one way ANOVA followed by Dunnett's multiple comparison test using the graph pad prism and a $P< 0.05$ is considered significant.

VI. RESULTS

Qualitative phytochemical analysis was carried out for MEOC as per the standard methods described by Prashant Tiwari *et al*[20] (2011).The results of the phytochemical analysis is summarized in **Table 1**.

TABLE 1

PHYTOCHEMICALS	MEOC
Alkaloids	Present
Flavonoids	Present
Tannins/phenols	Present
Saponins	Present
Sterols	Present
Carbohydrates	Present
Glycosides	Present
Proteins	Present
Amino acids	Present

Table 2: Shows the neuroprotective activity of methanolic extract of Oxalis Corniculata Linn on depression in albino mice.

Table 2. Forced swimming test

GROUP		NO. OF MICE	DURATION OF IMMOBILITY(SEC)	
			ACUTE STUDY	CHRONIC STUDY
GROUP A-Control(Normal Saline 10ml/kg)		5	95.63±0.98	97.39±0.13
GROUP B- Test drug 1 (MEOC 100mg/kg)		5	81.06±0.26[a]	78.08±0.28[a]
GROUP C- Test drug 2(MEOC 200mg/kg)		5	63.40±0.05[a]	61.32±0.10[a]
GROUP D- Standard drug(Imipramine 10mg/kg)		5	61.01±0.54[a]	60.76±0.20[a]
ONE WAY ANOVA	F		157.5	491.3
	Df		19	19
	P		<0.05	<0.05

Values are expressed as MEAN ± SEM (n=5). One Way ANOVA *followed by Dunnett's Multiple Comparison test is done between the groups.[a] $p < 0.05$ is considered significant.*

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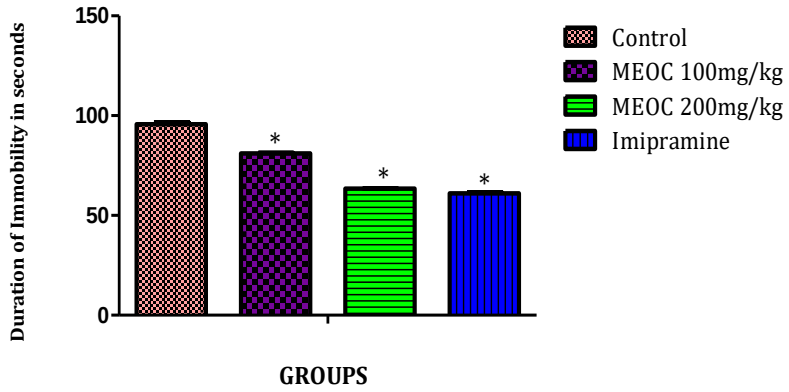
In the forced swimming test, the mice were judged to be immobile whenever they remained floating in the water in an upright position, making only small movements to keep its head above the water[23].

In the acute study, the mean for the duration of immobility in the control group, MEOC 100mg/kg, MEOC 200mg/kg and Imipramine 10 mg/kg were 95.63±0.98, 81.06±0.26, 63.40±0.05 and 61.01±0.54 respectively. Whereas for the chronic study, the mean for the respective groups were 97.39±0.13, 78.08±0.28, 61.32±0.10 and 60.76±0.20 respectively (Table 2).

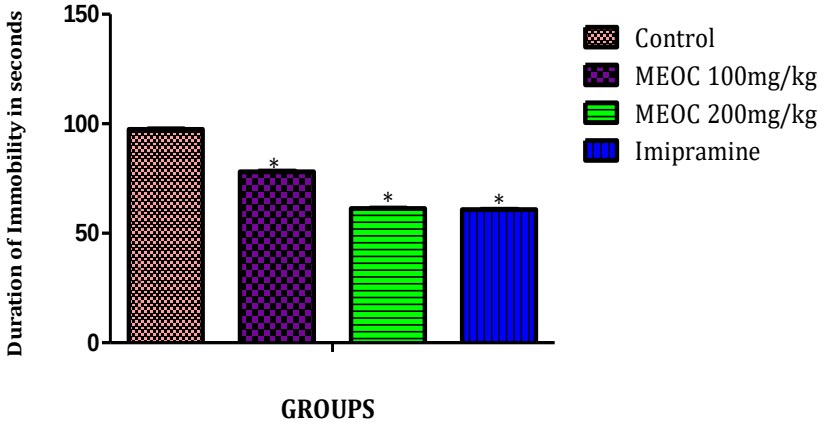
In the FST study, as seen from the table 2, both in the acute study and the chronic study, MEOC at doses of 100mg/kg & 200mg/kg, and the standard drug Imipramine, showed increase in the motor activity of the mice which elevated the depressed mood by decreasing the immobility time of mice. Both the doses of the extract and the standard drug Imipramine showed significant *decrease (p<0.05)* in the duration of immobility when compared with the control group. All the animals were healthy after the experiment and there were no deaths.

Figure 1 shows the Duration of immobility in Acute FST Study



p<0.05, when compared with the control group

Figure 2 ıows the Duration of immobility in Chronic FST study



p<0.05, when compared with the control group

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Table 3 shows the neuroprotective activity of methanolic extract of *Oxalis Corniculata Linn* on depression in swiss albino mice models.

Table 3 Tail suspension test

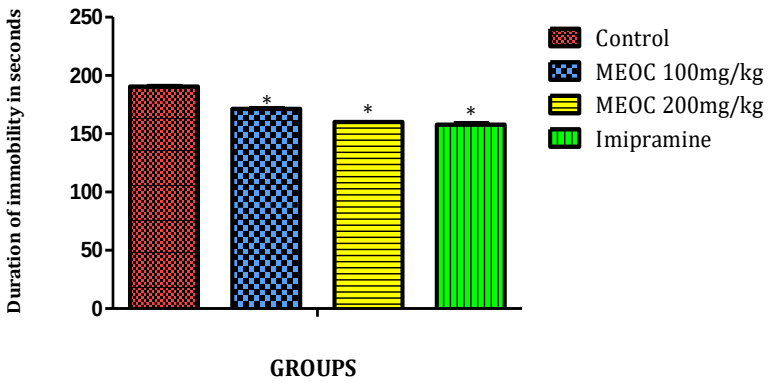
GROUPS		NO. OF MICE	DURATION OF IMMOBILITY	
			ACUTE STUDY	CHRONIC STUDY
GROUP A – Control (Normal Saline 10ml/kg)		5	190.5±0.41	195.20±0.23
GROUP B-Test drug 1 (MEOC 100mg/kg)		5	171.40±00.51[a]	170.80±0.12[a]
GROUP C-Test drug 2 (MEOC 200mg/kg)		5	160.10±0.01[a]	155.00±0.17[a]
GROUP D- Standard drug (Imipramine 10mg/kg)		5	157.80±01.14[a]	153.90±0.52[a]
ONE WAY ANOVA	F		512.6	3962
	Df		19	19
	P		<0.05	<0.05

Values are expressed as MEAN ± SEM (n=5). One Way ANOVA followed by Dunnett's Multiple Comparison test is done between the groups. p< 0.05 is considered significant.
[a]p<0.05, when compared with the control group

In the Tail Suspension test, all the mice attained an immobile posture after an initial period of vigorous activity. Mice were considered to be immobile only when they hung passively and were completely motionless[24].
In the TST, the parameters observed were the duration of immobility. The mean values for the duration of immobility for the control group, MEOC 100mg/kg, MEOC 200mg/kg and Imipramine 10mg/kg were 190.5±0.41, 171.40±00.51, 160.10±0.01 and 157.80±1.14 respectively. Whereas in the chronic study, the mean values for the duration of immobility for the respective groups were 195.20±0.23, 170.80±0.12, 155.0±0.17 and 153.90±0.52.

In the TST study, as seen from the table 3, both in the acute study and the chronic study, MEOC at doses of 100mg/kg & 200mg/kg, and the standard drug Imipramine, showed increase in the motor activity of the mice which elevated the depressed mood by decreasing the immobility time of mice. Both the doses of the extract and the standard drug Imipramine showed significant *decrease (p<0.05)* in the duration of immobility when compared with the control group. All the animals were healthy after the experiment and there were no deaths.

Figure 3 ıows the Duration of immobilty in Acute TST study



[*]p<0.05, when compared with the control group

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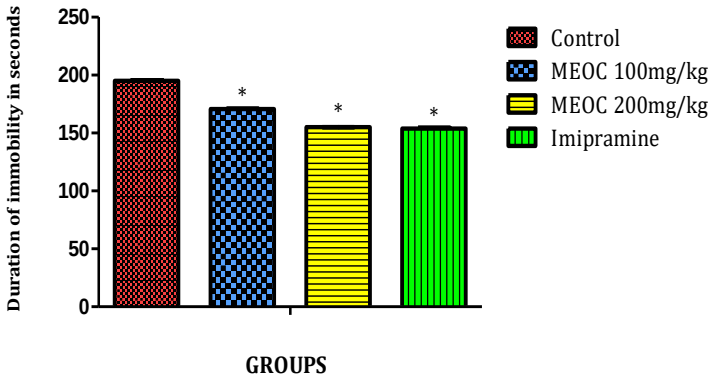
l **Figure 4** ws the Duration of immobility in Chronic TST study



****p<0.05, when compared with the control group***

VII. DISCUSSION

In the Forced swimming test and the Tail suspension test, duration of immobility was taken as the standard parameter to evaluate the antidepressant and thereby neuroprotective effect of MEOC on depression models.

In the forced swimming test, it was seen that both in the acute and chronic study, there was a significant decrease in the duration of immobility (*p<0.05*) in the two doses of test groups and the Imipramine group as compared with the control group. Both the doses of the extract and the standard drug Imipramine showed significant *decrease (p<0.05)* in the duration of immobility when compared with the control group.

In the tail suspension test, it was seen that both in the acute study and the chronic study, there was a significant decrease (*p<0.05*) in the duration of immobility in the two doses of MEOC treated group and the Imipramine treated group as compared with the control group. Both the doses of the extract and the standard drug Imipramine showed significant *decrease (p<0.05)* in the duration of immobility when compared with the control group.

In the present study, the antidepressant like activity associated with the acute and repeated administration (chronic) of MEOC in the two doses was assessed in the mice models of depression namely FST & TST. Both these tests are validated tests for antidepressant activity because the immobility produced in the animals can be reversed by many different classes of antidepressants[25].Thus MEOC by decreasing the duration of immobility on both the doses shows that it possesses antidepressant like activity and thereby neuroprotective property.

VIII. CONCLUSION

Oxalis corniculata Linn was used traditionally as anti-inflammatory, digestive, diuretic, antibacterial, antiseptic, in cardiopathy, hepatopathy, dysentery, diarrhea and skin diseases. It is also used in dyspepsia, wound healing, cancer, piles, dementia and convulsions. It was also reported that *Oxalis corniculata Linn* have hypoglycemic, antihypertensive, antipsychotic, nervous system stimulant & have chronotropic and inotropic effect. The methanolic extract of the whole plant of *Oxalis corniculata Linn (MEOC)* on the two experimental models, i.e Forced swimming test and the Tail suspension test showed significant neuroprotective activity on depression. Regarding the probable mechanism of action, it can be said that the extract was exerting the neuroprotective effect on depression , due to the presence of the phytochemicals and due to its potent antioxidant property. Now the phytochemicals that can be held responsible for the action were found to be flavonoids, saponins, tannins and polyphenols.

However, further research is necessary to gain a better understanding of its potential therapeutic action by isolating and identifying the phytochemicals responsible for the observed beneficial activities. There is also a need for further studies on a molecular level for the evaluation of neuroprotective effect of MEOC on depression on other animals and human beings that may provide definite data for its safety, efficacy, cost and also therapeutic use.

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