**ABSTRACT**

The objective of the present study is to investigate the antidepressant activity chloroform and ethanolic extract of leaves of *Antigonon leptopus* in Albino rat by tail suspension technique. The albino rats weighing about 100-150 gm were used and divided into seven groups, each groups containing six animals (n=6). Control group received distilled water (15 ml), Imipramine HCl (15mg/kg) for standard and test groups received chloroform and ethanolic extract of *Antigonon leptopus* in five different doses 50mg/kg, 100mg/kg, 150mg/kg, 200mg/kg and 250mg/kg per orally separately. They were evaluated for antidepressant effect by tail suspension model, after 30 minutes for ethanolic extract and after 60 minutes of drug administration. The immobility time was noted for five minutes for each rat in all groups. The results were analyzed by ANOVA followed by Dunnet’s multiple comparison test, p<0.05 was considered significant. The ethanolic extract at 100mg/kg and 150mg/kg and chloroform extract at 150mg/kg and 200mg/kg exhibited best result in tail suspension method, but the both extract possess significant activity at all test dose level. Present studies were suggested that *Antigonon leptopus* leaves exhibited good antidepressant properties and this would lead some support for its use in traditional medicine practice.

**KEYWORDS:** *Antigonon leptopus* leaves, Albino rat, Tail suspension technique, Antidepressant activity, Chloroform extract, Ethanolic extract.

**INTRODUCTION**

World Health Organisation reported that depression is a social and medical problem in all over world about 340 million people suffer from it.[1] Depression is observed to be
psychologically, symptomatically and biologically heterogeneous. Psychiatric disorder is also a cause of death and it is observed that between 10 and 20 million people suicide every year.[2] Depression is a common mental health problem that can range from mild to severe depressive disorder. It was characterized by loss of interest of pleasure, depressed mood, retardation of thinking and activity, low self worth or feeling guilty, loss of energy, loss of appetite, disturbed sleep, feeling of suicidal ideas.[3-5] There are several categories of drugs that are used as antidepressant for the treatment, often frequently produce side effects and patient face problems in tolerating these drugs is the prime factor to the noncompliance. The synthetic drugs which are frequently prescribed is especially tricyclic antidepressants produce drowsiness, emotional blunting, anxiety, restlessness, confusion, akathisia, hypersensitivity, dizziness, disbalance in weight and appetite, weakness, sweating, muscle twitches, sexual dysfunction, tachycardia, hypotension, nausea and vomiting and rarely irregular heart rhythms. The Selective Serotonin Reuptake Inhibitor exhibited an array of side effects like agitation, headache, nausea, diarrhoea and sexual dysfunction. Therefore it is essential that to the development of new molecules which have similar therapeutic with negligible or no side effects.[6-7] Hence, our aim for the treatment of the depression was to explore the potential of the plants.[8]

Therefore, our study focused on analysis of antidepressant activity of *Antigonon leptopus* in laboratory animals.[9] *Antigonon leptopus* is belonging to the flowering plant family Polygonaceae is a tender perennial vine. It is the native of Mexico and commonly found in the Caribbean, America, Tropical Asia and Africa and also known as Mexican creeper or coral vine.[10-11] According to previous study report various studies of these plant exhibited anti-thrombin[12], anti-diabetic[13], analgesic and anti-inflammatory[14], hepatoprotective[15], anthelmintic[16], anti-microbial[17], lipid peroxidation inhibitory activity[18] and traditionally it is used for the treatment of asthma, liver and spleen disorder[19], cough and throat constriction[20], reduce swelling and it’s leaves tea preparation are used to treat hypertension, diabetes and menstrual pains.[21] From the previous studies of this plant it is not found to any report on the antidepressant properties of *Antigonon leptopus*, therefore the present study was undertaken to analyze the possible antidepressant activity of ethanol and chloroform extract of *Antigonon leptopus* leaves by using tail suspension model in rat.
MATERIAL AND METHODS

Plant Material

The mature and fresh leaves of Antigonon leptopus were collected from Azamgarh city, Uttar Pradesh, India during 4 Oct 2013. There are to prepare the herbarium and botanical identification was done by Dr S.L. Gupta, a Scientist ‘E’ and Head of Office, Botanical Survey of India, C.R.C., Allahabad- 211002.

Preparation of extract

The leaves of the plants were washed with water, dried well under shade and prepare coarse powder that pass through meshes no.40. The fine powder was extracted by using soxhlet apparatus (a solvent system extraction by using ethanol and chloroform separately) at 40ºC to 60ºC and after 72 hr it was filter from Whatman filter paper and extracts were concentrated by using vacuum under pressure by rotator flash evaporator. The final product was kept at room temperature in a vacuum desiccator until analysis.

Animals

Either sex, 3-5 month old, procured from disease and weight around 150-200 gm collected from Pharmacy College Azamgarh, Uttar Pradesh, were used in experiment. With alternating light-dark cycle of 12 hr each in room where they housed and had free access to food and water. The rats were acclimatized for 7 days to the laboratory circumstances. The rats were kept under fasting to overnight and water ad libitum. The experimental protocol approved from the Institutional Animal Ethical Committee (IAEC) of Committee for the purpose of control and supervision of experiments on animal (CPCSEA).

Drugs and Chemicals

There are the following drugs and chemicals were used for the experiment: Imipramine (Ranbaxy Pvt Ltd. Mumbai), ethanol (Feicheng Jinta Machinery Co., Ltd.), Chloroform (Sd Fine-Chem. Limited Mumbai), Sodium chloride (Ranbaxy Fine Chemicals, New Delhi) Acetic acid (Ranbaxy Fine Chemicals, New Delhi).

Acute Toxicity Study

Acute toxic class-limit test dose guideline 425of Organisation for Economic and Cultural Development (OECD) was used for acute toxicity studies. All groups of animals were recorded within the 24 hr duration for percentage mortality and observed for further 14 days
for any sign for delayed toxicity. The ethanolic extracts of leaves of *Antigonon leptopus* at the doses of 2 and 5 mg/kg orally did not show any lethal dose effects.[22-24]

**Phytochemical screening**

Freshly prepared both ethanol and chloroform extracts was liable to phytochemical screening for observation of constituents by using a standard protocol.[25]

**Experimental Animals and treatment regimens**

Before one day of the experiment, the animals were divided randomly into control, standard and experimental groups (n=6). The first group (Group I) served as control group and receive vehicle, distilled water. The second group (Group II) has served as reference standard. Five groups (Group III, IV, V, VI and VII) served test groups and received chloroform and ethanolic extract of *A. leptopus* leaves (CEAL and EEAL, respectively) separately at five different doses such as 50, 100, 150, 200 and 250 mg/kg per orally. On the basis of our preliminary screening these five doses were selected.[26]

**Experimental protocol**

Tail suspension test was first given by Steru.et.al. is commonly used animal behavioural model for screening of antidepressant-like activity in the rat or mice. So the present study was based on this model with some modification. For adaptation of laboratory condition, animals were transported from their housing colony to laboratory in their own cages before 1-2 hr. Animals were individually hung on the edge of the shelf 50 cm by above the floor by the using of adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for 5 min by using stop-watch. Animal was considered to be immobile when they hung passively and completely motionless. The changes in immobility were studies after 30 min of administration of extracts, standard Imipramine and vehicle. The test was conducted dim light and noise free room.[27-28]

**Statistical Analysis**

All data was recorded and expressed as Mean ± SEM values were presented in the table I and II for ethanol and chloroform extract respectively. The response time were analysed by means of one-way analysis of variance (ANOVA) followed by Dunnet’s Test, using Graphpad Prism 6 Demo software. The result ware regarded as statistical significant at p-value < 0.05.
# RESULT

Table I: Anti-depressant effect of EEAL by tail suspension method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Pre Treatment</th>
<th>I (0.30 hr)</th>
<th>II (1.40 hr)</th>
<th>III (2.50 hr)</th>
<th>IV (4.0 hr)</th>
<th>V (5.10 hr)</th>
<th>VI (6.20 hr)</th>
<th>VII (7.30 hr)</th>
<th>VIII (24.0 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50</td>
<td>57.51±1.49**</td>
<td>56.75±2.25**</td>
<td>62.69±2.31**</td>
<td>56.44±1.56**</td>
<td>61.69±2.31**</td>
<td>56.54±1.46**</td>
<td>44.49±1.51**</td>
<td>51.54±1.46**</td>
<td>51.59±1.41**</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>58.99±3.01**</td>
<td>47.48±0.52**</td>
<td>55.50±1.50**</td>
<td>55.65±2.35**</td>
<td>50.60±2.40**</td>
<td>50.75±2.25**</td>
<td>45.60±1.70**</td>
<td>46.45±1.55**</td>
<td>51.70±2.30**</td>
</tr>
<tr>
<td>III</td>
<td>150</td>
<td>57.72±2.28**</td>
<td>51.57±1.43**</td>
<td>52.41±0.59**</td>
<td>53.46±1.54**</td>
<td>50.41±1.59**</td>
<td>44.36±0.64**</td>
<td>48.31±0.69**</td>
<td>54.36±0.64**</td>
<td>56.51±1.49**</td>
</tr>
<tr>
<td>IV</td>
<td>200</td>
<td>54.53±1.47**</td>
<td>52.48±1.52**</td>
<td>51.37±0.53**</td>
<td>55.37±0.67**</td>
<td>50.42±1.58**</td>
<td>49.37±0.67**</td>
<td>41.42±1.58**</td>
<td>48.37±0.63**</td>
<td>51.52±1.48**</td>
</tr>
<tr>
<td>V</td>
<td>250</td>
<td>56.44±1.56**</td>
<td>53.98±3.02**</td>
<td>51.33±0.67**</td>
<td>54.38±1.62**</td>
<td>40.43±1.57**</td>
<td>49.48±1.52**</td>
<td>54.33±0.67**</td>
<td>46.38±0.62**</td>
<td>42.43±1.57**</td>
</tr>
<tr>
<td>Std.</td>
<td>15</td>
<td>70.51±2.49**</td>
<td>50.43±1.58**</td>
<td>55.45±1.55**</td>
<td>49.47±1.53**</td>
<td>51.59±2.40**</td>
<td>53.40±1.60**</td>
<td>47.42±1.58**</td>
<td>54.54±2.46**</td>
<td>50.56±2.44**</td>
</tr>
<tr>
<td>Cont.</td>
<td>15</td>
<td>86.85±3.15**</td>
<td>84.47±1.53**</td>
<td>88.39±0.61**</td>
<td>79.41±1.59**</td>
<td>82.53±2.47**</td>
<td>76.44±1.56**</td>
<td>78.46±1.54**</td>
<td>81.58±1.42**</td>
<td>82.50±1.50**</td>
</tr>
</tbody>
</table>

All values are expressed in mean ± standard error mean (n=6).

All data were found to be significant at 5% level of significance where **p<0.05.

Table II: Anti-depressant effect of CEAL by tail suspension method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Pre Treatment</th>
<th>I (0.30 hr)</th>
<th>II (1.40 hr)</th>
<th>III (2.50)</th>
<th>IV (4.0 hr)</th>
<th>V (5.10 hr)</th>
<th>VI (6.20 hr)</th>
<th>VII (7.30 hr)</th>
<th>VIII (24.0 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50</td>
<td>58.50±1.45**</td>
<td>56.68±1.32**</td>
<td>55.58±1.42**</td>
<td>44.46±0.54**</td>
<td>42.43±0.57**</td>
<td>49.38±0.62**</td>
<td>57.35±0.65**</td>
<td>57.51±1.49**</td>
<td>47.35±0.65**</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>56.82±2.18**</td>
<td>56.62±1.38**</td>
<td>55.46±0.54**</td>
<td>60.64±0.36**</td>
<td>60.73±2.27**</td>
<td>62.67±1.33**</td>
<td>54.56±1.44**</td>
<td>51.52±1.48**</td>
<td>47.46±0.54**</td>
</tr>
<tr>
<td>III</td>
<td>150</td>
<td>58.46±0.54**</td>
<td>55.99±3.01**</td>
<td>42.46±0.63**</td>
<td>47.46±0.54**</td>
<td>56.54±1.46**</td>
<td>40.44±0.56**</td>
<td>40.47±0.53**</td>
<td>40.48±0.57**</td>
<td>54.37±0.63**</td>
</tr>
<tr>
<td>IV</td>
<td>200</td>
<td>54.64±1.36**</td>
<td>46.73±2.27**</td>
<td>41.37±0.54**</td>
<td>49.48±0.52**</td>
<td>42.45±0.55**</td>
<td>49.4±0.66**</td>
<td>53.58±1.42**</td>
<td>47.54±1.46**</td>
<td>55.58±1.42**</td>
</tr>
<tr>
<td>V</td>
<td>250</td>
<td>54.61±1.33**</td>
<td>53.46±5.4**</td>
<td>50.57±1.43**</td>
<td>54.59±1.41**</td>
<td>45.49±0.51**</td>
<td>43.47±0.53**</td>
<td>47.59±1.41**</td>
<td>57.56±1.44**</td>
<td>49.49±1.51**</td>
</tr>
<tr>
<td>Std.</td>
<td>15</td>
<td>70.51±2.49**</td>
<td>50.43±1.58**</td>
<td>55.45±1.55**</td>
<td>49.47±1.53**</td>
<td>51.59±2.40**</td>
<td>53.40±1.60**</td>
<td>47.42±1.58**</td>
<td>54.54±2.46**</td>
<td>50.56±2.44**</td>
</tr>
<tr>
<td>Cont.</td>
<td>15</td>
<td>86.85±3.15**</td>
<td>84.47±1.53**</td>
<td>88.39±0.61**</td>
<td>79.41±1.59**</td>
<td>82.53±2.47**</td>
<td>76.44±1.56**</td>
<td>78.46±1.54**</td>
<td>81.58±1.42**</td>
<td>82.50±1.50**</td>
</tr>
</tbody>
</table>

All values are expressed in mean ± standard error mean (n=6).

All data were found to be significant at 5% level of significance where **p<0.05.
Figure I: Effect of EEAL in Immobility time

Figure II: Effect of CEAL in Immobility time

Phytochemical Screening
The preliminary phytochemical evaluation of *Antigonon leptopus* leaves exhibited the presence of different phytoconstituents such as alkaloids, steroids, carbohydrates, tannins, terpenoids, saponin glycosides and flavonoids glycosides.

Acute Toxicity study
Acute oral toxicity studies revealed the nontoxic nature of the plant extract of *A. leptopus*. There was no any toxic manifestation observed at dose of 2000mg/kg and any death up profound to dose of 5000mg/kg in rat.
Tail Suspension Test
The responses of control and all extracts at different doses were compared. The results were found to be significant at 5% level of significance where P value<0.05. The effect of ethanol and chloroform extracts were more pronounced after 30 and 60 minutes respectively at all test doses. It was observed that ethanol extract at 100mg/kg and 150mg/kg and chloroform extract at 150mg/kg and at 200mg/kg possess highly significant reduction in immobility time when compared to control in a dose dependent manner. Similarly, the animals treated with standard drug (Imipramine HCl, 15mg/kg) exhibited significant decrease in immobility time as expected. The p values for ethanol at 100mg/kg and 150mg/kg are 0.0008 and 0.0009 after 30 minutes and chloroform extract at 150mg/kg and 200mg/kg are 0.0001 and 0.0004 after 60 minutes, respectively.

DISCUSSION
The present research work was subjected to evaluation of the antidepressant activity of chloroform and ethanolic extracts of Antigonon leptopus in rat. Primarily, the extract was subjected to preliminary phytochemical investigation and acute oral toxicity studies. The phytochemical evaluation of the plant leaves extract showed the presence of different phytoconstituents such as alkaloids, tannin, steroids, carbohydrates, terpenoids, saponins glycosides and flavonoids glycosides as per standard procedure. Plant leaves extract did show no any lethal effect and complete absorption of the drug through GIT was observed. The effect of EEAL and CEAL were investigated for its antidepressant activity by using most widely used animal models for this activity is tail suspension model. In this model, EEAL at 100mg/kg and 150mg/kg and CEAL at 150mg/kg and 200mg/kg, p.o. exhibited significant increase in the motor activity of rat which elevate depressed mood by decreasing immobility time of rat. The parameter which is observed in this model is immobility time of rat. Agents that decline immobility time lead to increase in the motor activity of rat which inhibits depression created by tail suspension model of rat. In the current study, ethanol extract at 100mg/kg and 150mg/kg and chloroform extract at 150mg/kg and at 200mg/kg has exhibited a significant dose dependent activity. It shows that increase in the dose of the drug is directly proportional to decrease in the immobility time threshold and permit good percentage safety as compared to control group. The standard drug Imipramine HCl (15mg/kg) had good percentage protection, similarly. Imipramine was a category of tri-cyclic antidepressant drug. Tri-cyclic antidepressant eases depression by affecting naturally existing chemical massengers (neurotransmitters) that are used to communicate between the brain.
The finding from the present investigation indicate that EEAL and CEAL possesses significant antidepressant property as shown by mitigating effects on different experimentally induced stress models in rats and mice.

CONCLUSION
This means the tail suspension test is more sensitive and better reflect the stress of the depression. Our present study confirmed that both EEAL and CEAL have the antidepressant activity as its significantly reduce the immobility time and increase the exploratory behaviour during depression in animal models.

REFERENCE
11. Raju AJS, Raju VK, Victor P, Naidu SA; Floral ecology, breeding system and pollination in Antigonon leptopus L. (Polygonaceae), Plant species Biology, 2001; 16: 159-164.


