EVALUATION OF ANTI CONVULSANT AND ANTI OXIDANT ACTIVITY OF SELECTED MEDICINAL PLANT

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ABSTRACT

Objective: Cuscuta reflexa is greatly valued plant reported in Indian traditional system of medicine for various ailments. In this study, anticonvulsant and antioxidant activity of ethanol extract of stems (EES) of Cuscuta reflexa Roxb. was investigated. Method: Protective effects were evaluated in pentylentetrazole (PTZ) (80 mg/kg, s.c.) induced seizure models in adult albino mice (200 mg/kg and 400 mg/kg) using diazepam as a standard. The antioxidant activity was evaluated by non enzymatic glycolysisation of haemoglobin. Result: The onset and duration of seizures (tonic-clonic convulsions), mortality rate and number of mice with and without convulsion within the observation period were noted in PTZ induced seizure models. EES showed anti oxidant activity. the result obtained indicated that ethyl acetate fraction has more anti oxidant activity than benzene and chloroform eluent. The detailed chemical nature of the active principles responsible for anti oxidant activity is not known. However preliminary phytochemical screening had confirmed
the presence of flavanoids, alpha tocopherol and rutin were used as the standard antioxidant compounds. EES at the dose of 400 mg/kg, exhibited maximum delay in onset of convulsions (6.44 min in PTZ induced seizures) in the model with increased latency period. The suppression of seizures by EES (400 mg/kg) might be observed due to enhanced gamma amino butyric acid neurotransmission in PTZ induced animals. Good free radical scavenging activity and considerable amount of flavonoids and coumarins in the extracts might have contributed in protection offered with good anticonvulsant effects in both the models. This study justifies the multilevel therapeutic uses of stem of Cuscuta reflexa in Indian system of medicine.

**KEYWORDS:** CPCSEA, FDA, PTZ, DZP, GABA, IAEC, WHO.

**INTRODUCTION**

People tend to rely on traditional and other forms of complementary and alternative medicine for chronic conditions which do not respond well to conventional or modern drug treatments. Among these are neurological disorders such as anxiety, pain and epilepsy. Centuries before the advent of modern medicine, synthetic chemistry and the pharmaceutical industry, virtually all medicines came from plants. These medicinal plants have been an important source for the discovery of novel bioactive compounds which served and continue to serve as lead molecules for the development of new drugs. Aspirin, atropine, scopolamine, taxol, theophylline, tubocurarine, vincristine and vinblastine are a few examples of such invaluable therapeutic tools for today’s physician’s. Epilepsy is one of the major neurological disorders affecting approximately 0.8% of the population. There has been considerable progress in the pharmacotherapy of epilepsy over the last few decades, including the introduction of new antiepileptic drugs such as felbamate, lamotrigine, etc. However, current drug therapy of epilepsy is complicated by side-effects, teratogenic effects; long term toxicity and about a third of patients are refractory to pharmacotherapies. Furthermore, there is currently no drug available which prevents the development of epilepsy e.g. after head trauma and all currently available AEDs drugs are synthetic molecules. Medicinal plants used for the therapy of epilepsy in traditional medicine practice possess promising anticonvulsant activities in animal models of anticonvulsant screening and these can be an invaluable source for search for new antiepileptic compounds. Majority of epilepsy patients rely on medicinal plants for therapy. For example, a sample in Nigeria found 52% of epilepsy patients using some form of traditional medicine. Also, the use of traditional medicine and medicinal herbs is currently
enjoying a renaissance in popularity in the West as well, and in fact, it is the primary form of medicine in many parts of the world. Epilepsy in particular is a condition where traditional healers are very critical in providing treatment in the rural settings. Considering the great reliance on traditional medicinal plants for treatment of diseases and the potential for drug discovery, it becomes relevant to search for potent, effective and relatively safe plant medicines as well as to scientifically validate success claims about plants already in use by traditional medicine practitioners.\textsuperscript{[1]}

Epilepsy is a major neurological disorder characterized by recurrent, spontaneous brain seizures or convulsions and its prevalence in developing countries is generally higher than in developed countries. Epilepsy is the second most common neurological disorder after stroke and it is estimated that approximately 0.8% of the population is affected by some form of epilepsy. Recent studies suggest an increased risk of dying and a greater proportion of deaths that are epilepsy-related in Africa as high as a six-fold increase in mortality in people with epilepsy. This is higher than the two-to-three fold increase reported in 6 developed countries. Though not clear, the reasons for this gap might be due to social deprivation. Recent data suggest that people from socio-economically deprived backgrounds in developed countries are more likely to develop epilepsy. This neurological disorder is viewed as a shameful disorder and has severe social implications in African communities as it carries a stigma. Sufferers are often shunned and discriminated against with respect to education, employment and marriage.

Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose-related and chronic toxicity that involve virtually every organ system. Moreover, all the currently available AEDs have potential for adverse effects on cognition and behavior. The practice of poly pharmacy in the therapy of epilepsy that has doubtful background increases the risk of side effects and drug interactions. It can be said that all problems with the current AED therapy of epilepsy are more prevalent in underdeveloped countries due to lack of facilities for proper diagnosis, treatment and monitoring of serum levels of AEDs. Another critical issue associated with currently available AEDs is recent clinical and experimental data that strongly suggest that AED therapy does not alter the course or natural history of epilepsy and though AEDs suppress the seizures, they may not affect the underlying disorder. Only a very few AEDs have been shown to be anti-epileptogenic including valproate and phenobarbitone and levetiracetam but these are not
well substantiated. There is pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs which are not only anticonvulsant but also anti-epileptogenics that either prevent epilepsy or alter the natural course.

**Pathophysiology of epilepsy**

A variety of different electrical or chemical stimuli can easily give rise to a seizure in any normal brain. The epileptic seizure always reflects abnormal hypersynchronous electrical activity of neurones caused by an imbalance between excitation and inhibition in the brain. Neurones are interconnected in a complex network in which each individual neurone is linked through synapses with hundreds of others. A small electrical current is discharged by neurones to release neurotransmitters of synaptic levels to permit communication with each other. More than hundred neurotransmitters or neuromodulators have been shown to play a role in neuronal excitation. However, the major excitatory neurotransmitter in the brain is L-glutamate and the major inhibitory neurotransmitter in the brain is gamma-amino butyric acid (GABA). An abnormal function of either of these could result in a seizure. An excited neurone will activate the next neurone whereas an inhibitory neurone will not. A normal neurone discharges repetitively at a low baseline frequency, and it is the integrated electrical activity generated by the neurones of the superficial layers of the cortex that is recorded in a normal electroencephalogram. If neurones are damaged, injured or suffer electrical or metabolic insult, a change in the discharge pattern may develop. In the case of epilepsy, regular low-frequency discharges are replaced by bursts of high-frequency discharges usually followed by periods of inactivity. An epileptic seizure is triggered when a whole population of neurons discharges synchronously in an abnormal way. This abnormal discharge may remain localized or it may spread to adjacent areas, recruiting more neurons as it spreads.

**Role of GABA and glutamate in the pathogenesis of epilepsy**

It is important to emphasize the role of neurotransmitters especially, γ-amino butyric acid (GABA) and glutamate in epileptogenesis, since they are the major inhibitory and excitatory transmitters in the central nervous system, respectively, and the fact that generation of seizures has been attributed to imbalance between excitatory and inhibitory neurotransmission in epileptic brains.

GABA plays an important role in regulation of neuronal excitability and impairment of GABA function produces seizures. Compounds that enhance GABA-mediated inhibition are convulsants. GABA exerts its major inhibitory effect via GABAA receptor (which is a
ligand-gated ion channel) by increasing neuronal membrane conductance for chloride ions causing membrane hyper-polarization resulting in reduced neuronal excitability and most rapid inhibition in brain. GABAA receptor is target for many important neuroactive drugs including antiepileptic drugs benzodiazepines and barbiturates. GABAA receptor consists of five subunits that form a chloride ion channel. The subunits consist of various subtypes and pharmacological studies have shown that individual subunits and subtypes confer different sensitivities to agents acting on GABAA receptors. It is postulated that exposure of GABA to postsynaptic receptors for a brief period of time results in generation of Inhibitory Post-Synaptic Currents (IPSCs). GABAA receptor-mediated miniature IPSCs play important physiological role in preventing the development of neuronal hyper excitability. Decrease in GABAA from receptor-mediated IPSCs is observed in cells from hippocampus of animals with chronic experimental epileptic seizures and humans with chronic intractable temporal lobe epilepsy.

AIM AND OBJECTIVE

Aim
The main aim of the study is to determine the anti convulsant and anti oxidant activity of the medicinal plant Cuscuta reflexa.

Objective
To evaluate the anticonvulsant and anti oxidant activity in a scientific manner using experimental model. Therefore the present study is undertaken with the following objective.

The work is carried out in the following phases:
1. Collection and authentication of the plant part
2. Preparation of extract of Cuscuta reflexa by successive extraction technique.
3. To study the acute toxicity for determination of LD50 of the extract in mice.
4. To determine the anti convulsant and anti oxidant activity of the plant Cuscuta reflexa.

Main Objectives of the Study
1) Phase-I
   i. Preparation of extracts using different solvents and testing the anti-oxidant activity of the different compounds of Cuscuta reflexa.
2) Phase-II
   i. To evaluate Anti-convulsant activity in Penteleteratzole induced seizures in mice.
3) Phase-III
   i. To study the anti convulsive role of stems of Cuscuta reflexa against experimentally induced convulsions.
   ii. Present study is undertaken to evaluate the anti convulsant activity of 90% ethanolic extracts of Cuscuta reflexa plant

MATERIALS AND METHOD

Plant collection and Authentication
The whole plant of Cuscuta reflexa had been collected from the medicinal garden of Sri Krishna Chaithanya college of Pharmacy, Chittoor District, Andhra pradesh, India. The plant was identified and authenticated by the Botanist Dr. K. Madhava Chetty, Assistant Professor, Department of botany, Sri Venkateswara University, Tirupathi.

Plant Preparation and Extraction
The leaves were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to macerate with ethanol for 72 hours at a temperature of 50-60 ºC. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum desiccators until further use.

Maceration Process
Procedure
As indicated in the Pharmacopoeia the process consists of the following: Place the solid material with whole menstrum in close vessel and allow stand for 7 days shaking occasionally. Strain presses the mark and mix the liquid obtained. Clarify by subsidence or filtration. This process is normally used for the preparation of tincture or extract and menstrum is usually alcoholic, hydrochloric (in case of tincture) or may be aqueous. Drug is kept with the menstrum for a long a period. The process is carried out at ambient temperature. At the end of process the mark is either pressed or menstrum is decanted depending upon the nature of drug to be extracted in the process. Depending upon the type of drug to be extracted by maceration two different methods is adopted. The types of drug to be extracted by maceration are either organized drugs or unorganized drugs. Organized drugs are either the parts of plant like roots, seeds, barks, etc. which have got the cellular structure. They contain the alkaloids glycosides.
Assessment of antioxidant activity EES of cuscuta reflexa

Evaluation by Non Enzymatic Glycolysisation of Haemoglobin Method

Shade dried leaves of Cuscuta Reflexa was extracted with petroleum and ethanol using soxhlet apparatus. The extracts were concentrated to dryness in vacuo. The ethanolic extract was subjected to silica gel column chromatography and eluted with benzene, chloroform and ethyl acetate. Further fractionation of ethyl acetate fraction using silica gel column and elution with benzene ethyl acetate afforded C6 H6 EtOAc (1:2) EBE -1 and C6H6 EtOAc (1:8) EBE-2.

Since non enzymatic glycolysation of haemoglobin is an oxidation reaction, an anti oxidant is expected to inhibit this reaction. The degree of glycolysation of haemoglobin in vitro can be measured colorimetrically. Haemoglobin 5g/100ml in 0.01M phosphate buffer (pH 7.4) was incubated in presence of 2g/100ml concentration of glucose for 72 h in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1ml glucose solution, 1ml of haemoglobin solution and 1 ml Gentamycin (20mg/100ml) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature. The degree of glysolysation of haemoglobin of fractions and their absence were measured colorimetrically.

Result of anti oxidant activity of Cuscuta Reflexa are summarized in the (Table no.4).

The result obtained indicated that ethyl acetate fraction has more anti oxidant activity than benzene and chloroform eluent. The detailed chemical nature of the active principles responsible for anti oxidant activity is not known. However preliminary phytochemical screening had confirmed the presence of flavanoids, alfa tocopherol and rutin were used as the standard antioxidant compounds.

Assessment of Anticonvulsant Activity of EES of Cuscuta Reflexa

Selection of Animals and Drugs

A. Animals

Healthy adult albino mice weighing between 170- 200 g were selected for the study and housed in well ventilated propylene cages in the animal house of Institute of Pharmacy, Hyderabad, India. Animals were maintained at 25 ± 2 °C, 50-60 % RH and kept under natural photoperiodic condition with free access to food and water. During the period of experiment the animals were fed with the standard diet (M/S Pranav Agro Tech Ltd. Vaghodiya,
Vadodara) and water ad libitum. They were kept for one week in laboratories before the experiments for acclimatization to the laboratory conditions. The study was conducted after approval from the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy. Animal ethics guidelines and good laboratory practice guidelines were followed and precautions were taken to minimize pain and discomfort to the animals.

B. Drugs
Pentylenetetrazole (Sigma, USA, 80 mg/kg. i.p), Diazepam (Calmpose inj. Ranbaxy, India. 4 mg/kg. i.p), were used in this study. The drugs were dissolved in water for injection and administered in a volume of 5 ml/kg to mice.

Acute toxicity studies
The acute toxicity study for ethanolic extract of *Cuscuta reflexa* roxb. was perform using albino swiss mice. Animals were fasted overnight prior to the experiment and mention under standard condition. Extract of drug administered orally in increasing dose and the animals were continuously observed for 24 hours. All animal at the end were safe without any toxic sign up to the dose of 2000 mg/kg that dose was taken as maximum tolerable dose. The 1/10th & 1/5th dose of maximum tolerable dose was 2000 mg/kg; on that basis 200 mg/kg and 400 mg/kg were selected as dose level. Doses were calculated for all animals on the basis of body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I</td>
<td>Vehicle 0.9% saline (1ml/kg)</td>
</tr>
<tr>
<td>Groups II (positive control)</td>
<td>Diazepam (4mg/kg op) +PTZ</td>
</tr>
<tr>
<td>Groups III</td>
<td>Ethanolic extract of <em>Cuscuta reflexa</em> (200 mg/kg, p.o.) once daily for 7 days +PTZ</td>
</tr>
<tr>
<td>Groups IV</td>
<td>Ethanolic extract of <em>Cuscuta reflexa</em> (400mg/kg, p.o) once daily for 7 days +PTZ</td>
</tr>
</tbody>
</table>

Method of Evaluation of anti convulsant activity
The mice were randomly divided into four different groups with 6 mice (n = 6) in each group for PTZ induced model (Table No. 1). Group I was served ad normal control and was administered with vehicle, Group II with Diazepam (4mg/kg, p.o) and served as standard. Group III & IV with two doses of stem extract (low and high dose).
Two different doses of EES were administered orally using oral gastric lavage tube one hour prior to the administration of the inducers viz. PTZ (80 mg/kg, s.c.). The animals were observed for 1 hr by placing in a separate cage. The onset of seizure and duration of tonic-clonic convulsions were recorded and the number of animals convulsing or not convulsing within the observation period were noted. The latency for development of convulsions in mice and mortality rate were also noted. The ability of the plant extract to prevent or delay the onset of convulsions was taken as an indication of anticonvulsant activity. The doses were decided on basis of literature review wherein these dose levels have been screened for other pharmacological activities of the extracts of same plant including the evaluation of anti-diabetic activity of the roots and stems of Cuscuta reflexa at the dose levels i.e. 200 and 400 mg/kg body weight in albino mice. The toxicity studies have also been reported for aqueous and ethanol extract of stem of EES of Cuscuta reflexa.

**Statistical analysis**

Data were expressed as mean ± S.E.M using Graph pad prism 5. Statistical significance between normal control and diseased control groups was tested using one way ANOVA followed by Post-hoc Dunnett’s t-test.

**RESULTS**

**Table no. 2: Results of Phytochemical Screenings of Extract of C. Reflexa.**

<table>
<thead>
<tr>
<th>Phytochemical screening</th>
<th>Direct extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test name</td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
</tr>
<tr>
<td>Molischs test</td>
<td>+</td>
</tr>
<tr>
<td>Barafoeds test</td>
<td>-</td>
</tr>
<tr>
<td>Seliwanoffs test</td>
<td>-</td>
</tr>
<tr>
<td>Test for pentoses</td>
<td>-</td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
</tr>
<tr>
<td>Dragendorffs test</td>
<td>-</td>
</tr>
<tr>
<td>Mayers test</td>
<td>-</td>
</tr>
<tr>
<td>Wagners test</td>
<td>-</td>
</tr>
<tr>
<td><strong>Amino acid</strong></td>
<td></td>
</tr>
<tr>
<td>Ninhydrins test</td>
<td>-</td>
</tr>
<tr>
<td>Millions test</td>
<td>-</td>
</tr>
<tr>
<td><strong>Test for waxes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Test for sterols triterpenoids</strong></td>
<td></td>
</tr>
<tr>
<td>Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td><strong>Test for starch</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Test for flavanoids</strong></td>
<td></td>
</tr>
<tr>
<td>Shinoda test</td>
<td>+</td>
</tr>
</tbody>
</table>
Table no. 3: Acute toxicity Study of Cuscuta reflexa.

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Dose</th>
<th>Average weight of the animal in grams Before After Treatment Treatment (1st day)(3rd day)</th>
<th>Signs of Toxicity</th>
<th>Effects observed</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>EECR</td>
<td>5 mg/kg</td>
<td>179 185</td>
<td>No signs of toxicity</td>
<td>No effect</td>
<td>Nil</td>
</tr>
<tr>
<td>EECR</td>
<td>50 mg/kg</td>
<td>170 179</td>
<td>No signs of toxicity</td>
<td>No effect</td>
<td>Nil</td>
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<td>No signs of toxicity</td>
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</tr>
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<td>EECR</td>
<td>1000 mg/kg</td>
<td>183 189</td>
<td>No signs of toxicity</td>
<td>No effect</td>
<td>Nil</td>
</tr>
<tr>
<td>EECR</td>
<td>2000 mg/kg</td>
<td>165 174</td>
<td>No signs of toxicity</td>
<td>No effect</td>
<td>Nil</td>
</tr>
</tbody>
</table>

EECR – Ethanolic extract of Cuscuta Reflexa.

Anti-Oxidant Activity

Table no 4: Anti-Oxidant Activity of Different Fractions of Cuscuta Reflexa Extract.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Final concentration of test compounds ( /ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>EB</td>
<td>Nil</td>
</tr>
<tr>
<td>EC</td>
<td>2</td>
</tr>
<tr>
<td>EBE-1</td>
<td>7</td>
</tr>
<tr>
<td>EBE-2</td>
<td>15</td>
</tr>
<tr>
<td>Alpha-Tocopherol</td>
<td>12</td>
</tr>
<tr>
<td>Rutin</td>
<td>11</td>
</tr>
</tbody>
</table>

The % inhibition of haemoglobin glycolysation was measured at three concentrations of benzene eluent EB, chloroform eluent EC and ethyl acetate fractions EBE-1 and EBE-2. The activity was compared with that of alpha tocopherol and rutin.

Anti-Convulsant Activity

Pentylenetetrazole (PTZ) Induced Convulsion Model Results of our study indicated that administration of EES at dose of 400 mg/kg body weight, one hour prior to injection of PTZ, significantly delayed the onset of convulsions (6.44 min, p<0.0001 which was found even higher than the standard group (7.89 min). However, it was observed that EES at the same
dose level were not able to decrease the duration of clonic and tonic convulsions. EES at the dose of 200 mg/kg body weight was also found to be active due to their ability to delay the onset of convulsion by 5.33 min respectively. Further, pre-treatment with EES was found to prolong the latency of convulsions induced by PTZ dose dependently and protected the animals from mortality. EES at the dose of 200 mg/kg and 400 mg/kg protected 41.90 % and 61.25 % of the animals while the standard antiepileptic drug, diazepam, offered complete protection to the animals from mortality.

**Table no. 5: Effect of Ethanolic Extract of Cuscuta Reflexa Roxb. on PTZ-induced seizures in Swiss Albino mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group number</th>
<th>Dose (Ml/Kg)</th>
<th>Onset of Seizures</th>
<th>Onset of Deaths</th>
<th>% Inhibition of Onset of Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>I</td>
<td>Saline water</td>
<td>3.01±0.121</td>
<td>4.23±0.324</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam Standard</td>
<td>II</td>
<td>2 mg/kg</td>
<td>7.89±0.498</td>
<td>11.45±0.0909</td>
<td>89.69</td>
</tr>
<tr>
<td>Test Compound</td>
<td>III</td>
<td>Low dose (200 mg/kg)</td>
<td>5.33±0.231</td>
<td>7.86±0.990</td>
<td>41.90</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>High dose (400 mg/kg)</td>
<td>6.44±0.767</td>
<td>9.51±0.936</td>
<td>61.25</td>
</tr>
</tbody>
</table>

(Values are expressed as mean ± S.E.M. n=6 *** p < 0.001, ** p < 0.01, * p < 0.05 compared with vehicle control ANOVA followed by Dunnet’s t- test).

**Fig no. 1: (Graph 1) Calculation of Antiepileptic activity of Cuscuta Reflexa Roxb. on PTZ- induced seizures in mice.**

**DISCUSSION**

Results of phytochemical analysis revealed that ethanolic extract of stems showed considerable amount of Steroids, flavanoid, tocopherol, rutin which may be responsible for observed antioxidant and anticonvulsant activities of EES. Phytochemical screening and
estimation of total flavanoid and steroids content showed considerable amount of flavanoids, tocopherol, rutin present in stems of this plant. This report suggests likely development of a mechanism behind the observed anticonvulsant activity of stems and the possible role of the flavanoids, tocopherol, rutin in anticonvulsant potential with underlying mechanisms like antioxidant activity. The anticonvulsant activity of EES of Cuscuta reflexa at two dose levels (200 and 400 mg/kg) was studied by PTZ induced seizure model. PTZ is a non competitive Gamma Amino Butyric Acid (GABA) receptor antagonist and works by inhibiting the activity of GABA at GABAA receptor. PTZ is the most popular and widely used chemically induced seizure model and it represents an effective model for human generalized myo-clonic and also absence seizure while strychnine model is considered valid for primary generalized seizures. Most of the anti-epileptic drugs act by enhancing GABA neurotransmission. In PTZ induced seizure model, PTZ produced clonic convulsions and lethality in mice, while pretreatment with EES at two different dose levels has resulted in to delayed onset of convulsions, prolonged latencies of tonic seizures and reduction in lethality.

In Ayurvedic medicine, the Cuscuta plant is said to be useful in diseases of eye and heart. The chemical examination of the plant has been done by Agarwal and Dutt (1935). Some Pharmacological studies on this plant were conducted by G.S Singh and K.W. Garg (1973). Their research studies are found to have anti histamines action in this plant. Some recent studies show that the following chemical constituents are identified i.e. Quercetin, Cuscutine, and Cuscutamide etc. Cuscuta’s seed and stem are highly medicinal values. The seeds are used for carminative and anodyne. The stem is purgative. Dixit et al reported that hair growth activity of this plant stem through the periodic transformation of hair follicle from telogen to anagen phases. Some in vitro studies are indicated antioxidant activity of the plant stems. This plant extracts are very close and identical in magnitude and comparable to that of standard antioxidant compounds used. Another one article reported that in vitro studies of free radicals scavenging activity may be to Phenolic compounds in Cuscuta reflexa extract. A cold infusion of the seeds is given as a depuration and carmination is pains and aches of the stomach. Seed poultice can also apply locally for pains. The stems in decoction are useful in constipation, flatulence, liver complaints and bilious affection. Reinvestigation of the chemical constituents of the stem of c.reflexa was under taken by M.K.Jain and R.K.Miahra in 1963 who successively extracted the dried stem with petroleum ether and alcohol. The petroleum ether extract on careful chromatography afforded a white solid identified as beta
sitosterol reported earlier by Gopinath et al, while Kaempferol and Bergenin were obtained from the alcoholic extract of the plant.

These studies were in complete agreement with the report of Patil Amol, 2009. Maragenin a triterpenoid from crude petroleum ether of C.reflexa has been isolated and its chemical nature was determined by US. Srinivastava and co workers. In the year of 1992 AGR. Nair and G.Thiripurasundari isolated 6,7- dimethoxycoumarin (scoparone),6-hydroxy -7-methoxy -4-hydroxyl phenyl –coumarin (melanettin), quercetin and hyperoside from C. reflexa collected over Bougainvillea spectabilis. The carotenoid pigments of Cuscuta were characterized with the help of HPLC and chemical properties. Major carotenoids characterized were, Beta carotene, lycopene, rubixanthin, lutein, violaxanthin along the esters of Beta–cryptoxanthin lutein, rubixanthin and violaxanthin. The invitro antioxidant activity of Cuscuta reflexa stem extract has been investigated by S.B Yadav et al., 2000,by estimating degree of non enzymatic heamoglobin glycosylation measured colorimetrically at 440nm. The ethyl acetate fraction of ethanol extract showed higher activity tan the other fractions. The antioxidants activity of extracts is very close and identical in magnitude and comparable to that standard antioxidant compounds used. In recent research (2011) the antitumor activity of the chloroform and ethanol extracts of Cuscuta reflexa was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice at doses of 200 and 400 mg/kg body weight orally, respectively, while acute oral toxicity studies were performed to determine the safety of the extracts.

**CONCLUSION**

In present study, anti-oxidant activity anti-oxidant of ethanolic extract of stems of Cuscuta reflexa against seizures induced by PTZ. The observed antioxidant and anticonvulsant activities are due to the presence of considerable amount of flavonoids, Tocopherol And Rutin in the extract of stems. Increased oxidative load is directly implicated as seizures can cause imbalance in oxidant, antioxidant system of brain which leads to oxidation of lipids, DNA and protein ultimately resulting into neuro degeneration. Ethanol extract of stems of Cuscuta reflexa showed good anticonvulsant activity in PTZ convulsions may be through the involvement of GABAergic and glutaminergic transmission and through glycine inhibitory property. However, further studies are needed to develop the exact underlying mechanism of anticonvulsant action of possible constituents of the plant after isolation of bioactives. Thus results of our study showed promising anti-oxidant and anticonvulsant effects of ethanol
extract of stems against both the toxicants and provided a scientific claim to the usefulness of this traditional plant in neurological disorders like epilepsy.

BIBLIOGRAPHY


