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Phytochemical investigation and exploration of CNS depressant efficacy of ethanolic root extract of *Cyperus kyllinga* Endl.

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Sedative drugs such as diazepam, lorazepam, pentoberbitone, amphetamine derivatives etc., are known to have permanent side effects. However, addiction to such drugs, possibly out of depression, is not uncommon. Similar drugs from plant origin with same efficacy could be a better safe alternative. In this study, we evaluated ethanolic extract of roots of the *Cyperus kyllinga* Endl. (Nut grass) for central nervous system (CNS) depressant activity (sedative activity) at the dose 20 and 40 mg/kg body wt. when administered i.p. The standard drug used was diazepam. The CNS activity was assayed in two experimental models: locomotor activity using actophtometer and skeletal muscle relaxant test by Rota rod apparatus. The ethanolic extract of the *C. kyllinga* demonstrated depression of the CNS activities in the mice. Phytochemical preliminary screening of the root extract has revealed the presence of flavonoids, carbohydrates, phenolic compounds, steroids and saponin glycoside.

Keywords: Actophotometer, Ayurvedic, Depression, Herbal, Nut grass, Sedatives, Rota rod apparatus, Unani

Mental disorders, particularly anxiety and depression, have registered an alarming rise during the last two decades affecting more than 650 million people worldwide. Serious neurological and behavioral disorders make up to 13% of the global disease burden surpassing both the cardiovascular diseases and cancer. About 20% of the world's children and adolescents suffer from mental disorders and about 0.9 million people commit suicide every year[1-3]. Central nervous system (CNS) disorders include abnormalities in both physical and psychological domains. Many drugs used for the treatment of CNS disorder have side effects and thereby considered noncompliant[4,5].

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Anxiety is an unpleasant emotional experience of daily living characterized by a series of apprehension, uneasiness or impending distress this feeling is usually associated with changes in autonomic nervous system and behaviour and it affects 1/8th of the total population worldwide[6,7]. The drugs that are used in the treatment of anxiety relieve the symptoms and offer palliative relief of temporary nature[8]. Currently available antipsychotics are associated with variety of autonomic, endocrine, allergic, haematopoietic, and neurological side effects. As a result, there is a high prevalence of usage of complementary and alternative medicines for treatment of psychiatric disorders. Growing acceptance and popularity of herbal medicine and complementary therapeutics worldwide has encouraged researchers to look for therapeutic lead compounds from the ancient systems for therapy, i.e. Ayurveda, Siddha and Unani which can be utilized for development of new drugs. Various compounds from different plant species have been demonstrated for their pharmacological effectiveness as therapeutic products for the treatment of neurological disorders in a variety of animal models[9-11].

Cyperus kyllinga (Fam. Cyperacaea) commonly called Nut grass and locally "Nirbisi", is a tufted perennial weed, 5-45 cm tall, with short, horizontal creeping rhizome, 1-2 mm in diameter and cornered by orate – lanceolate scales, internodes variable in length[12]. It possesses various medicinal properties and used to treat many diseases in indigenous system of Ayurvedic and Unani medicine[13]. Phytochemical investigations of *C. kyllinga* have confirmed that the ethanolic extract of its rhizome possesses flavonoids, triterpenoids and glycosides whereas the petroleum ether extract was found to possess triterpenoids and glycosides[14,15]. The crude extract of this plant is commonly used in ayurvedic system to treat diabetes. The plant extract has also shown its potential applications as analgesic, antibacterial, anticancer, antidiarrheal, antihelminthes antioxidant, clotting, diuretic and hepatoprotective agent[16-20].

In the current investigation, we studied the effect of ethanolic extract of roots of *Cyperus kyllinga* on CNS activity in Swiss albino mice and compared with diazepam as standard and Tween 80 as control drugs. The preliminary phytochemical tests were also performed.

Materials and Methods

The plant was collected from the local fields of Durgapur, West Bengal, India and authenticated by BSI (CNH/71/2011/Tech.II/), Shibpur, Howrah, West Bengal, India. Diazepam (Ranbaxy laboratories Pvt. Ltd.), Tween 80 (Merck specialty Pvt. Ltd.) and all others chemical used were of analytical grade.

Preparation of ethanolic extracts

In brief, the roots were carefully washed under running tap water followed by sterile distilled water and dried at room temperature for 5 days, pulverized to a fine powder and stored in airtight bottles. The root powder was later extracted with absolute alcohol by soxhlet extraction procedure and then concentrated by distilling the solvent.

Preliminary phytochemical test of the extract

Qualitative chemical tests were conducted for the ethanolic extract of the roots of *C. kyllinga* to identify various phytoconstituents. Tests for carbohydrate, alkaloids, phenols, tannins, glycosides, flavonoids, glycosides and proteins were done[21].

Animals

Adult Swiss Albino mice, male and female weighing 20-40 g, were employed under standard laboratory conditions (temperature 24-28°C, humidity 60-70%, and normal light-dark cycle) in the study were obtained from the Animal House of the College. All animal experiments were carried out in accordance with the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Forest and Environment, Government of India, and the study was approved by the Institutional Ethical Committee (BCPSR/IEAC/2013/03).

Toxicity evaluation

The LD_{50} of the *C. kyllinga* ethanolic root extract was found to be 251.3014 mg/kg body wt. for i.p. According to OECD guidelines, it is considered as a LD_{50} cut off value. Dose selected for pharmacological studies by fixed dose methods are mentioned below. Ethanolic extracts of *C. kyllinga* roots used for CNS study were 20 and 40 mg/kg body wt. which were much lower than the LD_{50}[22].

Determination of CNS effect by noting locomotor activity

The animals were divided into four groups, each containing six animals, and the necessary measurements/readings were noted. Gr. I was injected with 1% Tween-80 as blank (1%Tween 80); Gr. II with diazepam (4 mg/kg) as standard; Gr. III & IV with the ethanolic root extract of *C. kyllinga* at dose of 20 (test 1); and 40 mg/kg (test 2), respectively. After 20 min of injecting the animals, the locomotor activity of each animal was noted by actophotometer (Digital Actophotometer, Bluefic Industrial and Scientific Technologies) for 5 min. The locomotor activity in actophotometer was also noted at 20 min, 40 min, 60 min, 80 min and 120 min after injection[23,24].

Determination of CNS effect by noting skeletal muscle relaxant activity

The experimental animals were divided into four groups and each group of animal contained six animals and all the animals were weighed and numbered accordingly. Basal reading on Rota rod of each animal of each group was noted at 20 rpm of the Rota rod. All the groups had the same treatment as done above. About 30 min after the injection of test material, control vehicle or the standard the animals were placed on Rota rod moving at an rpm 20. The fall off time from the rotating rod was noted. The assessment was repeated after 60, 90, 120 and 150 min. The difference in the fall off time from the rotating rod between the basal and the treated mice (standard- control/diazepam/extract) was taken as an index of muscle relaxation[23,24].

Results and Discussion

Phytochemical tests

The preliminary phytochemical evaluation of ethanolic root extract of *Cyperus kyllinga* revealed the presence of flavonoids, carbohydrates, phenolic compounds, steroids, saponin glycoside. The result of phytochemical analysis was significant in ethanolic root extract. These observations (Table 1) clearly indicate that most of the bioactive compounds of *C. kyllinga* are present in its root.

Table 1 — Preliminary phytochemical screening of *Cyperus kyllinga*

Chemical test	Present/Absent
Carbohydrates	+
Alkaloids	−
Proteins	−
Steroid	+
Cardiac glycoside	−
Saponin glycoside	+
Coumarin glycoside	−
Anthraquinone glycoside	+
Flavonoids	+
Tannins and Phenolic compounds	+

CNS depressant effect by noting locomotor activity

Effect on locomotor activity of ethanolic extract of *C. kyllinga* @ 20 and 40 mg/kg was studied on actophotometer (Fig. 1). Both the doses of test sample have shown significantly depressed activity in CNS compared to that of control group. However, both the extract doses were found to be less significant when compared to the standard drug diazepam (Fig. 1). Between 20 and 40 mg/kg, it was found that CNS depressant activity proportionally increased with increasing the dose. It was also noticed that the maximum depression of both doses occurred between 60 and 80 min (Fig. 1). After 80 min, the mice started recovering due to the decreased action of drug represented in Fig. 1. Whereas, the effect of standard drug diazepam stayed for a longer time. This result concludes the sedative effect of *C. kyllinga* root extracts on animals.

CNS depressant effect by noting skeletal muscle relaxant activity

Skeletal muscle relaxant property was studied on Rotarod injecting ethanolic extract of *C. kyllinga* at the dose of 20 and 40 mg/kg to the mice. Both extracts showed significant depression in skeletal muscle when compared with control group as observed in Fig. 2. Additionally, these were found to be less significant in contrast to the standard drug diazepam in Fig. 2. On assessment of dose of 20 and 40mg/kg, it was observed that the skeletal muscle depressant activity enhanced with increased dose as depicted in Fig. 2. In 20 mg/kg dose, at 60 min the mice started recovering due to the decreased action of extracts whereas the effect of standard drug Diazepam was for a longer time (Fig. 2).

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs elucidate their action through GABAA, and hence, it is possible that ethanolic extract of *Cyperus kyllinga* root may act by potentiating GABAergic inhibition in the CNS via membrane hyper polarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extract[25]. In this study, locomotor activity measured by open field tests, showed that the extract significantly decreased locomotor activity which indicates it has CNS depressant activity. Diazepam, which was used to induce sleep in this study, acts at specific binding sites that are closely linked to γ-aminobutyric acid (GABA) receptors, the

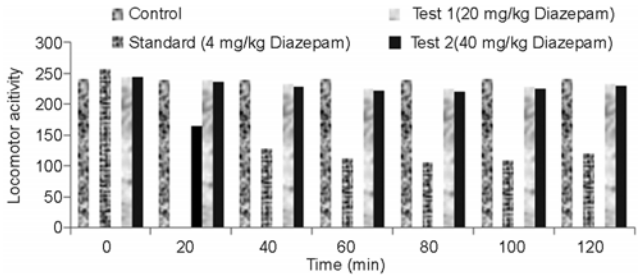

Fig. 1 — Comparison of CNS depression activity (using actophotometer) among the various doses of Blank, Standard, Test-1 and Test-2.

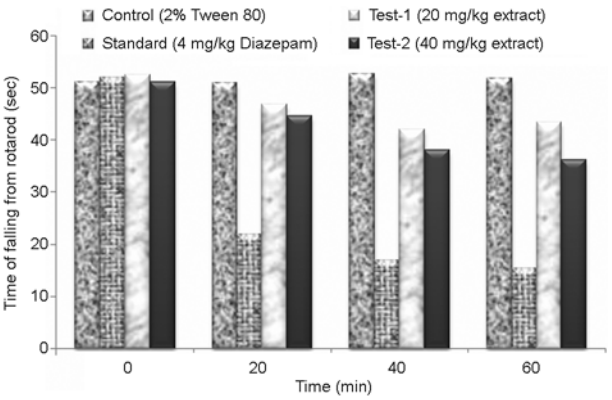

Fig. 2 — Comparison of skeletal muscle relaxant activity (using rotarod) among the various doses of Control, Standard, Test-1 and Test-2.

binding of benzodiazepines enhancing GABA-ergic transmission[26]. Phytochemical investigations also showed the presence of glycoside, flavonoids, saponins and tannins in the extract, which could be responsible for its CNS depressant activity[27]. The locomotor activity showed maximum depression for both doses between 60 and 80 min (Fig. 1), and after 80 min, the mice started recovering due to the decreased action of drug as represented in Fig. 1. Whereas, the effect of standard drug diazepam was for a longer time. This result concludes that the sedative effect of *Cyperus kyllinga* root extracts on animals. In the determination of CNS effect by noting skeletal muscle relaxant activity @ 20 and 40 mg/kg, it was observed that the skeletal muscle depressant activity enhanced with increase in dose as depicted in Fig. 2. In 20 mg/kg dose, at 60 min the mice started recovering due to cause of decreased action of extracts whereas the effect of standard drug diazepam was for a longer time (Fig. 2).

Conclusion

The experimental results conclude that ethanol extract of roots of the *Cyperus kyllinga* plant has CNS

depressant activity. But in contrast to standard drug i.e. Diazepam it was found to be less. Therefore, the plant extracts may be considered as CNS depressant for further study. Presence of flavonoids, saponins, tannins and steroids are useful in many CNS disorders[28]. Our study revealed the presence of flavonoids, carbohydrate, tannins, phenols, saponin in *Cyperus kyllinga* root. This study has established the central nervous system depressant properties of *C. kyllinga* root. Increase in locomotor activity is considered as an increase in alertness and decrease in locomotor activity indicated sedative effect. The present work includes further studies on isolation and characterization of the active components responsible for the CNS activity.

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