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Neuroprotective Activity of Saussrealappa (Clarke) on Experimental Animal Model

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Abstract: ***Neuroprotection refers to the strategies and relative mechanism able to defused the central nervous system against the neuronal injury. Depression is whole body illness which involves not only mood or emotion but also the physical body and thought process.There are two types of mental depression namely unipolar depression in which mood swings is common (about 75% of cases). The second type is bipolar depression (about 25% of cases) sometimes also called as endogenous depression. Many theories proposed to explain the mechanism of ageing at the molecular level the oxidative stress or free radical hypothesis has received wide support at the biochemical level oxidation. Neurodegeneration is a process involved in both neuropath logical conditions and brain ageing although the brain accounts for less than 2% of the body weight it consumes about 20% of the oxygen available through respiration. Many flavonoids extracted from nature plants have been reported to exert antidepressant like effect in animal studies.Saussurealappa (Compositae) is cultivated as a medicinal plant in the Himalayas. CostiAmari radix or costus root was an important item of Roman trade with India, and is believed to have been the dried root of Saussurealappa have reviewed extensively the medicinal importance. The animals treated with Saussurealappa extract produced dose dependent activity against neurological deficit, anti-depressant activity and Nootropic activity. Anxiolytic effect was not produced by the extract.***

Keywords: Nootropic activity, bipolar depression, oxidative stress, Anxiolytic activity

1. Introduction

According to world health report about 450 million people suffer from a mental or behavioral disorder .depression is expected to constitute the second largest source of global burden of diseases after heart diseases.1

Neuroprotection refers to the strategies and relative mechanism able to defused the central nervous system against the neuronal injury due to both acute /stroke or trauma and chronic neurodegenerative disorder s(e.g.: Alzheimer'sdisease, Parkinson'sdiseases).2

As complementary and alternative therapy herbal medicine or simple phytotherapy refers to the medical use of plant organs (leaves, stems, roots, flowers, fruits, and seeds) for curative properties Nutritional therapy is a healing systems using functional food and nutraceuticals as therapeutics.

Depression is whole body illness which involves not only mood or emotion but also the physical body and thought process. The symptoms of depression are intense feelings of sadness, hopelessness and despair as well as the inability to experience pleasure in usual activities changes in sleep patterns and appetite, loss of energy and suicidal thoughts.

There are two types of mental depression namely unipolar depression in which mood swings are always in the same direction and is common (about 75% of cases) non familial, clearly associated with stressful life events and accompanied by symptoms of anxiety and agitation.

The second type is bipolar depression (about 25% of cases) sometimes also called as endogenous depression shows a familiar pattern unrelated to external stress and usually appears in early life, results in oscillating depression and mania over a period of a few weeks.

Patients with depression have symptoms that reflect decrease in brain monoamine neurotransmitters, specifically nor epinephrine, serotonin and dopamine 500,000/year is diagnosed as suffering from depression a neurodegenerative disease is defines as a deteriation often irreversible of the intellectuals and cognitive faculties and it is generally associated with ageing and oxidative stress. Ageing is a complex physiological process that involves both morphological and biochemical changes occurring with passage of time in single cell and the whole organism.

Among the many theories proposed to explain the mechanism of ageing at the molecular level the oxidative stress or free radical hypothesis has received wide support at the biochemical level oxidation or reduction status leading to the production of partially reduced oxygen intermediates more reactive than molecular oxygen in its ground state including both radical and nonradical forms collectively termed as reactive oxygen species.

Neurodegeneration is a process involved in both neuropath logical conditions and brain ageing although the brain accounts for less than 2% of the body weight it consumes about 20% of the oxygen available through respiration

The brain is the most susceptible organ to oxidative damage the high amount of poly unsaturated fatty acids(PUFAS) present in neuronal membranes makes the brain tissues particularly susceptible to lipid per oxidation reaction resulting in the formation of cytotoxic aldehyde such as malondialdehyde (HAD) and 4-hydroxy noneal.

Many flavonoids extracted from nature plants have been reported to exert antidepressant like effect in animal studies. The antidepressant like effectof the hydroalcoholic extract obtained from aerial parts of Siphocampylus verticillatus, a Brazilian medicinal plant, was investigated in two models of depression in mice and against synaptosomal uptake of serotonin, noradrenaline and dopamine. The immobility times in the forced swimming test (FST) and in the tail suspension test (TST) was significantly reduced by the extract (dose range 100-1000 mg/kg, i.p.), without accompanying changes in ambulation when assessed in an open field [12].

The antidepressant like effect of the ethanol extract obtained from barks of tabebuia Avellaneda, a plant widely employed in folk medicine, was investigated in two predictive models of depression: forced swimming test (FST) and tail suspension test (TST) in mice. Additionally, the mechanisms involved in this antidepressant like action and the effects of the association of the extract with the antidepressant fluoxetine, desipramine and bupropion in the TST were investigated [14].

Plant Profile [18]:

Costus (Saussurealappa) belongs to the compositae family. In siddha medicine, it is known as Kostum. It is being cultivated in Kashmir and the Himalayan regions for its root.

Botanical name - Saussurea lappa, C.B.Clarke
Class - Dicotyledon
Sub-class - Gamopetalae
Series - Inferae
Cohort - Asterales
Family - Compositae

Root:
Root stout, often upto 0.6 m long and 0.3 m girth possessing a characteristic penetrating odour. The dried roots are very light thick, twistiform to cylindrical 7-15cm long and 1-5 cm thick grayish to brown and marked with longitudinal ridges. Texture very firm internally, lightish brown here and there sprinkled with resinous shiny granules.

Chemical Constituents:
Resinoids - 6%
Essential oil - 1.5%
Alkaloid named saussurine - 0.05%
Inulin - 18%
Resin - 6%
Potassium nitrate - Trace
Fixed oil and sugars - Trace

Uses:
In siddha system of medicine it is said that it can be used for ailments of eyes, stomach, neck, jaws, tongue, mouth and also be used for fever, edema, wheezing(dyspnoea), haemorrhoids, spermaturia. In Siddha system costus root has been used as in the form of powder (Chooranam), decoction, Saussurea lappa (Compositae) is cultivated as a medicinal plant in the Himalayas [19].

Costi amari radix or costus root was an important item of Roman trade with India, and is believed to have been the dried root of Saussurea lappa (Moeslinger et al., 2000). Pandey et al., 2007 have reviewed extensively the medicinal importance of Saussurea lappa.

Of the six compounds reported here in, compound1 is reported for the first time in the roots of Saussurea lappa. However, it was reported earlier from the aerial parts of the plant Messerschmidia sibrica (Boraginaceae). Compounds 2-6 are reported earlier from Saussurea lappa but their isolation protocols are very much different from that of the current study [20].

2. Materials and Methods

Plant Material:
70% Ethanolic root powder extract of Saussurea lappa

Animals: Swiss albino mice.

Standard drug: Diazepam

Preparation of extract:
Powdered drug was extracted by percolation process. Powdered drug was percolated using 70% ethanol for 72hrs. Then the solution was filtered and concentrated using heating mantle. Finally the ethanolic extract was subjected for further studies.

Results: Phytochemical Evaluation.

S. no.	Phytochemical compound	Ethanolic Extract
1	Alkaloids	Positive
2	Carbohydrates	Negative
3	Glycosides	Positive
4	Proteins	Negative
5	Volatile oils	Positive
6	Fats and fixed oils	Positive
7	Steroids	Positive
8	Flavanoids	Positive

3. Methodology

Assessment of Neurological Deficit Using Rotarod Apparatus

Aim: To Study the effects of diazepam on motor coordination in mice using Rotarod apparatus.

Rationale: Diazepam causes neurological deficit and causes motor in-coordination. As untreated mouse trained to remain on the rod rotating at the speed of 20 revolutions/minute for 5 minutes falls down at an earlier time when treated with diazepam. The effect is suggestive of neurological deficit induced by diazepam.

Requirement: Rotarod, diazepam
Procedure: Mice were trained to remain on rotating rod for 5 minutes. They were divided into four groups with 6 animals in each group. The animal were treated as follows
Group 1: Control 10ml/kg (distilled water)
Group 2: Diazepam 5mg/kg
Group 3: Extract 200mg/kg

Group 4: Extract 400mg/kg
After 30 minutes they were placed individually on the rotating rod moving at the speed of20 revolutions/minute and the latency to fall from the rotating rod was measured [44].

Assessment of Antidepressant Activity Using Forced Swim

Aim: To study effect of diazepam administration on duration of immobility in forced swim test in mice.

Rationale: Diazepam is a benzodiazepine, $GABA_a$ receptor agonist which increases the chloride ion influx produces hyperpolarisation hence used as antidepressant. The untreated animals were trained for 10 minutes and then animals were treated with the drug and after half an hour the animals were tested [45].

Requirements: Forced swim test apparatus, diazepam.

Procedure: The animals were trained for 10 minutes and treated according to the treatment groups and after half an hour they were forced to swim in the apparatus individually for 10 minutes and the immobility time was recorded. The mouse was judged immobile if it ceased struggling and remained floating motionless in water making only those movements necessary to keep its head above water. Reduction in the duration of immobility was considered as antidepressant like effect [46].

Assessment of Anxiolytic Activity Using Elevated Plus Maze

Aim: To Study the Anxiolytic effect of diazepam using Elevated Plus Maze in Mice.

Rationale: Placing an animal in an unaccustomed position or a new environment induced anxiety in animals which alters its exploratory behavior. A mouse when placed on the elevated plus maze prefers to remain in the enclosed arm. The animal spends more time in the enclosed arm and the number of entries in the open arm is also increased. If the drug has Anxiolytic effect, then the mouse will spend more time in the open arm and the number of entries in the open arm shall also increase [47].

Reqiurement: Elevated plus maze and stop watch

Procedure: The animal became anxious when placed on an elevated surface. The platform should be elevated atleast 25cm from the ground. The elevated plus maze (EPM) was first described by Lister, (1987). The EPM consisted of two open arms (25x5 cm) crossed with two closed arms (25x5x20 cm). The arms were connected with a central square of 5x5 cm in a dimly illuminated room. The mice were divided into groups of 6 each and were treated according to the treatment groups. The mice were divided into groups of 6 each and were treated according to the treatment groups. The animals were placed individually in the center of the EPM facing the closed arm and the time spent in the open and closed arms was recorded for 5 min [48].

Assessment of Nootropic Activity Using Elevated Plus Maze

Aim: to study the effects of Piracetam on cognitive behavior using elevated plus maze.

Rationale: The previous exposure of an animal to the elevated plane induces fear and to avoid the feeling of fear the animal occupies a safe position in the elevated plus maze. The latency to reach the central position in the elevated plus maze is indicative of the learning ability of an animal. The animal is said to have learnt if the latency to reach the central platform is reduced. The drugs impairing memory delay the entry of animal in the central platform [49].

Requirement: Elevated plus maze.

Procedure: mice were placed individually at the end of an open arm of the elevated plus maze (EPM) facing away from the central platform. And the time required to reach the central platform (TL) was noted. On the first day, mice were allowed to explore the maze for 5 minutes after the measurement of TL. On the following day, mice received the vehicle or diazepam (5mg/kg) 30 minutes before the measurement of TL. The TL was expressed as inflection radiation (IR) using the formula used by Joiswal and Battacharya [50].
$IR = (L_1/L_0)/L_0$ where,
L_1 = is the TL on day one
L_0 = is the TL on day two

Anti-depressant activity
Anti depressant activity of ethanolic extract of Saussurea lappa (EESL) was evaluated by forced swim test apparatus. Immobilization of animals was taken as parameter during forced swim .Rats were treated with reference standard showed significant ($p<0.001$ decreased in immobilizationtime when compared with control and EESL treated with two different dose (200 and 400 mg/kg bw) were significantly ($p<0.001$) decreased in immobilization time which is equal to normal level. The effect was observed as dose dependent activity. The results were present in table 1.

Table 1: Anti depressant activity of ethanolic extract of Saussurea lappa

Treatment group	Drug	Immobilisation of rats in seconds
Group I	Vehicle saline 10 ml/kg	76±2.01
Group II	Diazepam 5 mg/kg	64± 1.06*
Group III	EESL 200 mg/kg	110±0.01*
Group IV	EESL 400 mg/kg	76±0.16

All values expressed in Mean ±SEM. One way ANOVA followed dunnet test * $p<0.001$ non-significant

Table 2: Neurological deficit study of ethanolic extract of Saussurea lappa

Treatment group	Drug	Average time of fall in seconds in minutes		
		0	30	60
Group I	Vehicle saline 10 ml/kg	150±0.01	148±0.26	201±2.13
Group II	Diazepam 5 mg/kg	160±1.02	81±0.16	79±1.87
Group III	EESL 200 mg/kg	143±0.34	206±1.13	296±1.13
Group IV	EESL 400 mg/kg	172±2.07	83±0.34	132±2.03

All values expressed in Mean ±SEM. One way ANOVA followed dunnet test * p<0.001 non-significant

Table 3: Nootropic activity of Saussurea lappa

Treatment group	Drug	Average time taken to reach the centre point	
		Before	After
Group I	Vehicle saline 10 ml/kg	11±0.02	11±0.01
Group II	Diazepam 5 mg/kg	16± 1.01	9±0.09*
Group III	EESL 200 mg/kg	17±0.34	11±0.05^{ns}
Group IV	EESL 400 mg/kg	16±2.06	8±0.1*

All values expressed in Mean ±SEM. One way ANOVA followed dunnet test * p<0.001 non significant

Table 4: Anxiolytic activity of ethanolic extract of Saussurea lappa

Treatment group	Drug	Number of entries in arm		Time spent in arm	
		Open	closed	Open	closed
Group I	Vehicle saline 10 ml/kg	----	----	---	-----
Group II	Diazepam 5 mg/kg	3± 0.1	1±0.01	20±0.01	4.8±1.03
Group III	EESL 200 mg/kg	1±0.01*	----	---	5±0.87
Group IV	EESL 400 mg/kg	1±0.1	----	----	5±1.6

All values expressed in Mean ±SEM. One way ANOVA followed dunnet test *

4. Conclusion

The animals treated with Saussurea lappa extract produced dose dependent activity against neurological deficit, anti depressant activity and Nootropic activity. Anxiolytic effect was not produced by the extract. In lower doses (200mg/kg) the extract produced neurological deficit, antidepressant activity and Nootropic activity. In higher doses (400mg/kg) the effects are opposed. The effects are compared with standard Diazepam and it is concluded that the lower doses of ethanolic extract of Saussurea lappa shows similar like Diazepam might be $GABA_A$ receptor agonist activity.