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PHARMACY INFOPEDIA
PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

Article

Evaluation of adaptogenic activity of various extracts of Breynia Vitis-idaea (burm.f) C. Fisher leaves by using swim endurance test

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ABSTRACT

The present study was under taken to study the Adaptogenic activity of ethanol, aqueous and ethyl acetate extracts of Breynia vitis-idaea (burm.f) c. fisher. (Euphorbiaceae) leaves. Adaptogenic activity of these extracts were studied using in-vivo Swim Endurance test and compared with the standard Fluoxetin. All the extracts showed significant increase in the swimming time. Adaptogenic nature of the extracts may be attributed to the presence of constituents such as alkaloids, glycosides, flavonoids and saponins in plant extracts. This study proven that the potential adaptogenic nature of the extract of Breynia vitis-idaea leaves.

Keywords: Breynia vitis-idaea, Adaptogenic, ethanol, aqueous, ethyl acetate extract

INTRODUCTION

Herbal medicines are a valuable as well as a precious gift from nature. They were existing even before human beings made their appearance on the earth. Wherever we are born we have around us herbs, shrubs, and plants useful to us [1]**.** It is gratifying to note that in India, the importance and relevance of herbal system (Ayurveda, Unani, Sidda) is increasingly being realised for the last few decades. It is apparent that the sleeping giant of ayurveda is finally waking up [2].

The term **adaptogen** is used by herbalists to refer to a natural herb product that is proposed to increase the body's resistance to stress, trauma, anxiety and fatigue. In the past, they have been called rejuvenating herbs, qi tonics, rasayanas, or restoratives. All adaptogens contain antioxidants, but antioxidants are not necessarily adaptogens and that is not proposed to be their primary mode of action [3].

As a Pharmacotherapeutic group adaptogens were recently defined as preparations that increased attention and endurance in fatigue, and reduced stress-induced impairments and disorders related to the neuro-endocrine and immune systems [4].

Impact on disease

Stress can significantly affect many of the body's immune systems, as can an individual's perceptions of and reactions to stress. The term psycho neuro immunology is used to describe the interactions between the mental state, nervous and immune systems, as well as research on the interconnections of these systems. Immune system changes can create more vulnerability to infection and have been observed to increase the potential for an outbreak of psoriasis for people with that skin disorder. [5].

ETIOLOGY OF DEPRESSION

Depression is caused by risk factors such as heredity, brain chemical, personality traits, medication taken, medical conditions, vitamin deficiencies, type of diets, *etc* cause depression in people [6].

i. Genetic factors

In depression, one theory suggests that a variant of the gene responsible for encoding the serotonin

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PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

transporter protein could account for early childhood experiences being translated in to an increased risk of depression through stress sensitivity in adulthood in bipolar disorder some genetic linkage has been food with chromosomal region 6q16-q21. Comparison of the risk of affective disorder in the children of both parents with an affective disorder show our time greater risk, and the risk is doubled in children with one parent with an affective disorder studies looking at twins have found fairly strong evidence for a genetic factor.

ii. Biochemical factors

deficiency of neurotransmitter amines in certain areas of the brain this theory has been developed to suggest that receptor sensitivity changes may be important, alternative propositions suggest a central role of acetylcholine arising from regulation of the cholinergic and nor-adrenergic neurotransmitter systems although many neurotransmitters may be implicated, the theory focuses on an involvement of the neurotransmitters nor-adrenaline, serotonin and dopamine this theory emerged from the finding that both mono amine oxidase inhibitors (MAOIS) and tricyclic antidepressants appeared to increase neurotransmitter amines ,particularly nor-adrenaline at important sites in the brain.

iii. Insomnia and sleep disorder

Studies estimate that 20% of people with insomnia suffer from depression and 90% of people with depression have insomnia .Although stress and depression are major causes of insomnia, insomnia may also increase the activity of the hormones and pathways in the brain that can produce emotion problems. [7].

iv. Environmental factor

Although environmental stresses can often be identified prior to an episode of mania or depression, a casual relationship between a major event in someone`s life and the development of an affective disorder has not been firmly established.

v. Endocrine factors

The endocrine system, particularly the hypothalamic-pituitary –adrenal (HPA) axis and the hypothalamic-pituitary –thyroid (HPT) axis, is felt to be implicated in the development of thyroidism and Cushing's syndrome has also been associated with changes in mood.

DIAGNOSIS OF DEPRESSION

A patient must exhibit either depressed mood or diminished interest on pleasure in usual activities and must have at least five some symptoms from the following:

1. Significant weight loss or weight gain, or decrease or increase in appetite.
2. Insomnia or hypersomnia .
3. Psychomotor agitation or retardation as observed by others.
4. Fatigue or loss of energy.
5. Feelings of worthlessness, or inappropriate guilt.
6. Diminished ability to think or to concentrate.
7. Recurrent thoughts of death, suicidal, ideation, suicide attempt or a specific plan for suicide. [8]

Rating scales

Two most commonly used rating scales are to assist with the assessment of the severity of the disorder.

Back depression inventory

Hamilton depression rating scale [9]

Dexamethasone suppression test

The dexamethasone suppression test is still used by some clinicians as an aid to diagnosis, but it must be considered as having limited value in practice. Bipolar disorder is frequently misdiagnosed, and consequently patients are often inappropriately treated. This test the administration of 1 mg of dexamethasone at 11 p.m. Which is said to coincide with the low point of cotisol secretion? It would be expected that normally dexamethasone would suppress the secretion of cortisol for about 24 hrs. Blood samples are taken following day 8 a.m., 4 p.m. and 11 p.m. if it is found that serum cortisol levels are elevated between 9 and 24 hrs. Then this taken as positive result, i.e., dexamethasone has failed to suppress normal cortisol secretion [10]

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ANIMAL MODELS TOSCREEN ANTI-DEPRESSANT ACTIVITY

***IN VITRO* METHODS**

1. Inhibition of [^{3}H]-nor epinephrine uptake in rat brain synaptosomes.
2. Inhibition of [^{3}H]-dopamine uptake in rat striatal synaptosomes.
3. Inhibition of [^{3}H]-serotonin uptake in synaptosomes.
4. Antagonism of p-chloramphetamine toxicity by inhibitors of serotonin uptake.
5. Measurement of β-adrenoreceptors stimulated adenylate cyclise.
6. [^{3}H]- Yohimbene binding to a_{2-} adrenoreceptors in rat cerebral cortex.
7. Monoamine oxidase inhibition: inhibition of type A and type B monoamine oxidase in rat brain synaptosomes

INVIVO METHODS

1. Forced swim test
2. Reserpine induced hypothermia
3. Apo morphine induced hypothermia in mice
4. Serotonin syndrome in rats.
5. Muricidal behaviour in rats
6. Tail suspension method
7. Compulsive gnawing in mice
8. Learned helplessness in rats.

Breynia vitis-idaea (Burm.F.) C. fisher. (Euphorbiaceae) is an evergreen, glabours tree or large shrub. Found in the genetic plain, western peninsula, China, India, Malay Peninsula and Sri Lanka.. Bark is yellowish gray, leaves are alternate dark brown when dry, and flowers are small, greenish yellow and dull red, purple or white berries. Root, leaves and bark are medicinal. Root decoction is used as mouthwash. A new sulphur-containing spiroketal glycoside, breynin I and a new terpenic glycoside, breyniaionoside E together with 10 known compounds, were isolated from the plant [11]

EXPERIMENTAL SECTION

Plant material: The leaves of *Breynia vitis-idaea* were collected from surrounding area of Maddur, Mandya District in Karnataka, India. The plant was identified and authenticated by Botanist Prof. Nagendra. T. (Specimen no: 594) Bharathi College, Bharathinagar, Mandya District, Karnataka, India. The leaves were dried under shade then pulverized into coarse powder by a mechanical grinder and used for extraction.

Extraction Process: About 200 grams of dry powder of leaf of *Breynia vitis-idaea* was extracted first with ethanol for 72 hrs. The powdered drug was dried and packed well in Soxhlet apparatus and extracted. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used. The marc left after the extract was dried and subsequently extracted with ethyl acetate. The extraction was continued up to 72 hrs. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used. The marc left after the extract was dried and subsequently extracted with water. The extraction was continued up to 72 hrs. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used. [12,13]

Experimental Animals

Albino mice (Wistar strain) of either sex weighing between 20-30 g were procured from the Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka. The animals were acclimatized for three months under laboratory conditions.

The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) (Approval no: **BCP/IAEC/PCL05/2013-14**) of Bharathi College of Pharmacy, Bharathinagara, Mandya. Studies were performed in accordance with the CPCSEA guidelines.

Acute oral toxicity study (LD_{50}) (OECD Guide line 423) [14]

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic co-operation of Development [OECD], received drafts guidelines 423, revised from committee for the

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PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

purpose of control and supervision of experiment on animals [CPCSEA]. Female albino wrester mice (20-30 g) were used for test. Groups of 3 mice each received a single dose of test drug 5, 50, 300 and 2000 mg/kg orally. While one group served as the control. They were observed for gross behavioural changes 1, 2, 4, 6, 24, 48 & 72 hours after drug administration and further observed till 14 days for any mortality. Gross pathological changes were also observed on the completion of the experiment.

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2 ºC). Lighting was controlled to supply 12 hrs of light and 12 hrs of dark for each 24 hrs period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level.

Cage side observations In acute toxicity study, the animals were observed prior to dosing. Thereafter, observations were made at every hour, for five hrs and then at 24 hrs and then every day for 14 days. All observations were systematically recorded, with individual records being maintained for each animal.

Signs recorded during acute toxicity studies: Direct observation parameters include convulsions, diarrhoea, lethargy, sleep and coma. Skin and eyes and mucous membrane, respiratory, circulatory, and autonomic and central nervous systems, somato-motor activity and behaviour pattern are the other parameters observed. The time of death, if any, was recorded.

Feed and water consumption and body weight measurement

The animals were monitored daily for mortality, feed and water consumption and changes in body weight for 14 days in acute toxicity study. Feed intake was calculated as g/animal/day. Water intake was calculated as ml/animal/day

Anti-Stress Screening (*In vivo* method)

Swim Endurance Test: [15]

Table no:- 1 Grouping of animals.

GROUP	TREATMENT
1	control
2	standard (fluoxetine)
3	aqueous extract of plant low dose
4	aqueous extract of plant high dose
5	ethanol extract of plant low dose
6	ethanol extract of plant high dose
7	ethyl acetate extract of plant low dose
8	ethyl acetate extract of plant high dose

The mice were divided into eight groups of five each as above and the treatment also given in different dose. Group I served as control and given an equal amount of vehicle alone; Group II received 50 mg/kg per orally of standard drug and Group III, V & VII received the BVIP extract 50 mg/kg as a low dose of aqueous, ethanol and ethyl acetate extracts per oral, respectively. And Group IV, VI & VIII received the plant extract 100 mg/kg as a high dose of aqueous, ethanol and ethyl acetate extracts per oral, respectively After 1 hr, the animals were put to swimming in Plexiglas cylinder (height 40 cm, diameter 10 cm) filled with tap water (temp 27 ^{o}C) to a height of 18 cm and were allowed to swim actively till exhausted and drowned, which was taken as the end point.

Swimming time for each animal was recorded. The mean swimming time for each group was then calculated.

Statistical analysis: all results of experiment were expressed as mean ± sem. Statistical analysis were carried out with Prism version-5.0 using ANOVA followed by Dennett's test ($P<0.05$).

RESULTS AND DISCUSSION

In the present study, *B. vitis-idaea* leaf parts were extracted with ethanol, ethyl acetate and water were analysed for presence of phytochemicals and subjected to *in-vitro* hepatoprotective activity. Phytochemical analysis showed that the presence of alkaloids, flavonoides, glycosides, and saponins in

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PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

both ethanol and aqueous extracts. Ethyl acetate extract shows presence of glycosides and flavonoides. This showed in table no-2.

Table -2: Phytochemical analysis of the extracts of *breynia vitis-idaea*.

Extracts	Alkaloids	Flavonoides	Glycosides	Saponins
Aqueous extract	+	+	+	+
Ethanol extract	+	+	+	+
Ethyl acetate extract	–	+	+	–

Effect on General Behaviour and Acute Oral Toxicity

Table no 3 : Behavioural parameters observed in mice

The following behaviour patterns observed in mice during acute toxicity study.

Sl. No	RESPONSES	AEOP	EEOP	EAEOP
1	Alertness	N	N	N
2	Grooming	A	A	A
3	Touch response	P	P	P
4	Activity	P	P	P
5	Pain response	P	P	P
6	Convulsion	A	A	A
7	Corneal reflux	P	P	P
8	Pupils	N	N	N
9	Urination	N	N	N
10	Skin colour	N	N	N
11	Lacrimation	A	A	A
12	Hyper activity	A	A	A
13	Weight	N	N	N
14	Death	A	A	A
15	Body tone	A	A	A
16	Diarrhoea	A	A	A
17	Body tremors	A	A	A
18	Food consume	N	N	N
19	Water consume	N	N	N

***N- Normal, *P-Present, *A-Absent**

All the animals received different doses of extracts showed no sign of any change in their gross behavioural patterns throughout the period of observation. There was no mortality up to a dose of 2 g/kg (2000 mg/kg) during the 14 days period of observation. However, a dose of 5 g/kg (5000 mg/kg) produced 10% mortality after 72 hrs of its administration. This clearly indicates that the 3 extracts of plant did not produce oral toxicity. So the therapeutic dose for the pharmacological evaluation by plant was 1/10th of the maximum tolerated dose which was then fixed to be 50 and 100 mg/kg of the experimental animal for the further studies.

Swim Endurance Test

Administration of the extracts of *Breynia vitis-idaea* leaves in following doses it shows increase in the swimming duration of the mice. Each group contains 5 animals.

Grouping of animals

Group I (control) → 10 ml/kg.p.o.,
Group II (Fluoxetine drug) → 50 mg/kg.p.o.,
Group III (AEOP low dose) → 50 mg/kg.p.o.,
Group IV (AEOP high dose) → 100 mg/kg.p.o,
Group V (EEOP low dose) → 50 mg/kg.p.o.,
Group VI (EEOP high drug) → 100 mg/kg.p.o.,
Group VII (EAEOP low dose) → 50 mg/kg.p.o.,
Group VIII (EAEOP high dose) → 100 mg/kg.p.o,

Where

AEOP -- aqueous extract of plant
EEOP -- ethanol extract of plant
EAEOP -- ethyl acetate extract of plant

Table 4 Effect of extracts of plant on swim endurance test

GROUP	DOSE (mg/kg)	SWIMMING TIME (MEAN ± SEM)
1	10 ml/kg	13.18 ± 0.005
2	50	19.06 ± 0.01 ***
3	50	15.53 ± 0.01***
4	100	18.11 ± 0.007***
5	50	15.22 ± 0.1**
6	100	18.00 ± 0.01***
7	50	14.31 ± 0.007
8	100	16.03 ± 0.005**

Statistical significance was determined by one-way ANOVA followed by Dunnet's t test. Values represent

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PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

Mean ± SEM of five animals per group. And P value is ***<0.001. Compare to control.

Figure :1

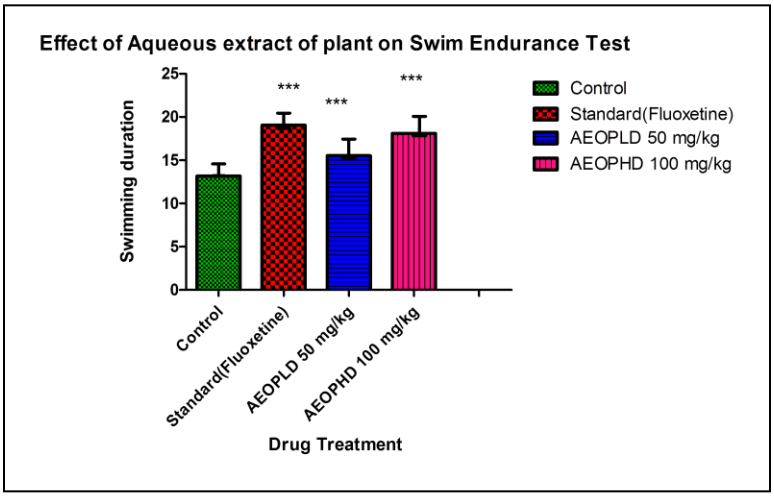
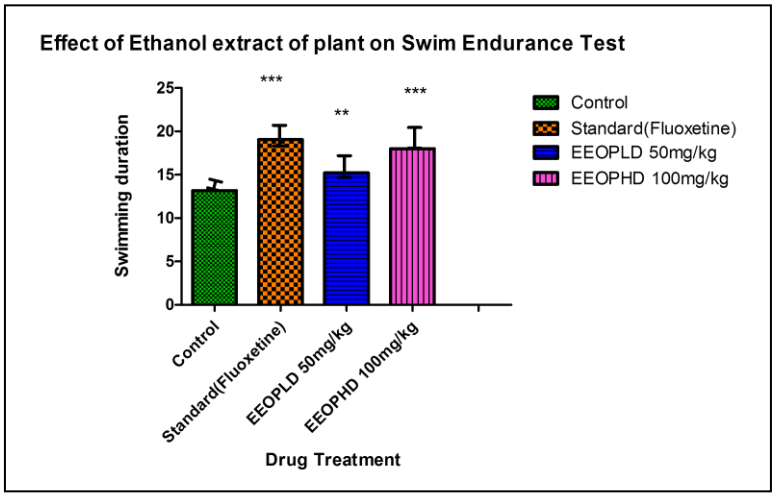


Figure: 2



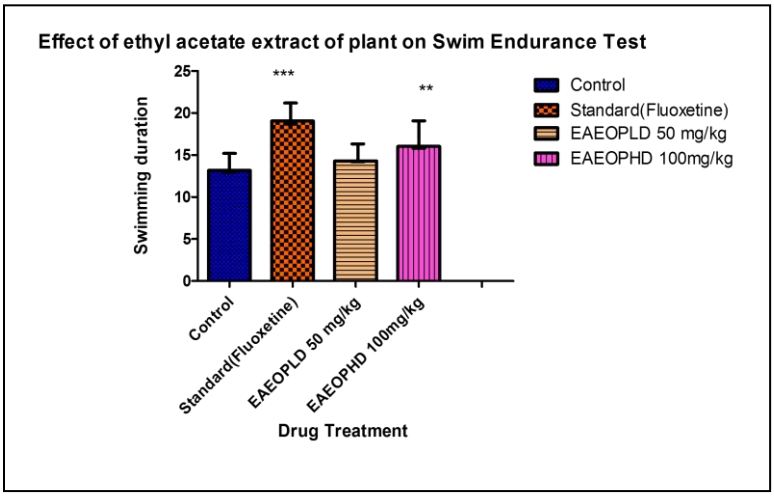


Figure: 3

On above swim endurance test following data's are noted.

The standard drug in different dose is produced significant increase and the mean duration of swimming time was high compared to control group. Plant leaf extracts Aqueous, ethyl acetate and Ethanol were administered at a dose of 50 and 100 mg/kg also produced significant increase in the swimming time versus control group. As shown in the above table no-4.

CONCLUSION

The activity performed was targeted the increase resistance power and the outcome of the results are highly encouraging and matching. Based on the result we concluded that aqueous, ethanol and ethyl acetate extracts of plant proves the anti-stressing action. The extracts were found to be nontoxic. Also we can use plant for wound healing.

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PRINT ISSN: 2394-6679 | E-ISSN: 2347-7881

Acknowledgement : The authors are thankful to parents for their support in all the way. Also to head of the Pharmacology department, Bharathi college of pharmacy, Mandya for guide and encouragement. The authors acknowledge Green chemicals company, Bangalore, India, for providing sample of plant extracts, Department of botany also acknowledged for his help in identification of plant.

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