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Pharmacology

Neuropharmacological study of *Humboldtia vahliana* Wight

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Original Research Article

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Abstract: The present study was aimed to investigate ethanol and aqueous extracts of barks of *Humboldtia vahliana* Wight (Caesalpiniaceae) for various neuropharmacological activities, such as sedative and hypnotics, anticonvulsants, antianxiety and antidepressants with estimation of brain neurotransmitter level by using animal models. Extracts were obtained by continuous hot extraction using solvents with different polarities. Two doses (200, 400mg/kg) of ethanol and aqueous extracts of *Humboldtia vahliana* Wight was orally administered for one time to evaluate sleep latency and sleep time in Phenobarbitone Sodium induced sleep in mice, maximum electric shock induced seizure for the assessment of anticonvulsant activity where time duration of various phases in epilepsy were noted. Open field test for antianxiety activity, in which number square crossed with respect to cut off time and in forced swim test, immobility time duration was monitored for antidepressant activity. The extract which had highly significant effect was subjected to HPTLC study. After administration of extracts, restored the altered parameters to normal when compared to positive control group. The ethanol and aqueous extracts showed no signs of mortality on mice in acute toxicity studies at 2g/kg. Administered extracts shortened the sleep latency and prolonged the sleep time in phenobarbitone sodium induced sleep, in EEHV (400mg/kg)(p.o) the sleep latency was 14±1.42 and the duration of sleep was 163.20±4.02. Similarly reduced the time of various phases of seizure in MES induced seizures especially extensor phase, the duration of time(sec) for flexion , extensor, clonus, stupor and recovery was 1.50±0.28, 10.0±0.40, 13.25±0.48, 31.5±0.64 and 96.0±0.71 for EEHV(400mg/kg)(p.o). The doses of extracts showed significant anxiolytic activity by reduction in locomotion in open field test as in EEHV(400mg/kg)(p.o) the duration if time(min) from 0, 30, 60, 90 and 120 was reduced as 45.0±1.29 , 34.01±1.47, 25.0±1.29 ,16.25±1.10, 3.50±0.65 and the dose of antidepressant activity increased the immobility time(min) of mice in forced swim test as 223.25±1.38 for EEHV(400mg/kg)(p.o). It also restored the brain neurotransmitter such as adrenaline and dopamine to more or less to normal level. HPTLC fingerprint revealed the presence of six peaks at 254 and 366nm respectively. The results revealed that ethanol extract of *H. vahliana* possesses significant effect on neurological disorder than aqueous extract. The ethanol extract at dose 400 mg/kg body weight was found to be more effective than lower dose. This effect is due to the presence of phytoconstituents in these plants like, flavonoids, saponins and phenolic compounds

Keywords: *Humboldtia vahliana* Wight, CNS, anxiolytic, depression, locomotion, neurotransmitters.

INTRODUCTION

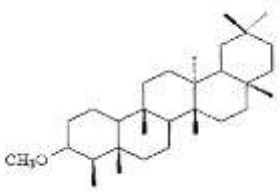
Neuropharmacology is the scientific study of the effects of drugs on the central nervous system. There are a number of drugs being used in the traditional medicine for treatment of various CNS disorders and presently many of these drugs are being explored scientifically to ascertain their CNS activities[1]. Drugs acting on central nervous system were among the first to be discovered by primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs produce specific physiological and psychological effects from the vast array of materia medica of the indigenous system, so many plants have been reported to have activities against CNS disorders and thus act as very useful remedies for the alleviation of human suffering [2]. Search for new molecules that act on the central nervous system and that can be used for therapeutic purposes is yet a challenge to the medical system [3].

In spite of the recent advances in understanding neurological disorders, there are still no effective therapies. Because in modern medicine

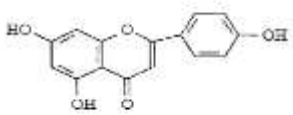
treatment is empirical symptom oriented and not disease specific. And also the currently available drugs which are in clinical use have low characteristic profile, with higher incidence of adverse effect for instance, antipsychotic drugs like fluoxetine, imipramine and antianxiety drugs like diazepam, alprozolam are used for many neurological disorders but these drugs produce severe adverse effects and withdrawal symptoms. Hence management of neurological disorders with agents devoid of any side effect is still a challenge to the medical system. This has led to an increase in the demand of natural products with neurological activity having lesser side effects. Thus there is a dire need for agents having neuroprotective and neuropharmacological activity [4].

Therefore, the investigations of novel pharmacotherapy from medicinal plants to prevent psychiatric illnesses and cognitive impairment have significantly progressed and obtained very much attention [5]. Indian flora and fauna is so vast that the Indian scientists are investigating plants, based on their use in traditional system of medicine, for the treatment of neurological disorders [6]. Many research showed that plant containing flavonoids, steroids and tannins are useful in many CNS disorders [7].

Humboldtia vahliana Wight (Caesalpiniaceous) is a moderate sized tree, bark are dark brown, mottled with white. The tree is endemic to the southern western ghats of Kerala and Tamil Nadu [8]. From the aerial parts of *Humboldtia vahliana* Wight, 3α-methoxy friedelan, β-sitosterol, sitosterol-3-0-β-D-glucopyranoside and 5.7.4'-trihydroxyflavone (apigenin) were isolated.



3α-methoxy friedelan



5.7.4'-trihydroxyflavone

The bark of the tree is used in biliousness, leprosy, ulcers and epilepsy[9]. To the best of our knowledge the neuro pharmacological effects of *Humboldtia vahliana* Wight were not been explored yet. In the light of literature survey and based on the knowledge of traditional use, this plant was used as an anticonvulsant in folk medicine practice. Therefore an attempt could be made to evaluate its neuro pharmacological parameters. The present study investigates for the first time various neuro pharmacological activities of Humboldtia vahliana Wight bark extracts.

MATERIALS AND METHODS

Plant material

The bark of *Humboldtia Vahliana* Wight (Caesalpiniaceae) was collected on 28/01/2015 at its beginning of blooming stage from Kerala and was identified and authenticated by Dr. A. G. Pandurangan, Scientist F & Head PS&ES Division. The voucher specimen (84381) was deposited in Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Pallode, Trivandrum, Kerala, and Department of pharmacognosy, Sree Krishna College of Pharmacy and Research Centre, Parassala, Trivandrum, Kerala.

Preparation of plant extracts

The shade dried barks was powdered and was extracted in Soxhlet apparatus consecutively using solvents with gradient polarities with, petroleum ether(60^0C) , benzene ($60\text{-}80^0$C) is used to determine the presence of certain chemical constituents in the extract, chloroform ($60\text{-}80^0$C), ethanol ($60\text{-}80^0$C) , and marc was macerated in water for 48hrs. Extracts were filtered through vacuum filter and the filtrate was concentrated in vacuum and evaporated. Ethanol extract of *H. vahliana* (EEHV) and aqueous extract of *H. vahliana* (AEHV) were used for animal study and was given per orally after dissolving it in water for injection.

Phytochemical Screening

Qualitative chemical test were conducted on benzene, chloroform, ethanol and aqueous extracts of *H. vahliana* to identify various phytoconsituents like flavonoids, alkaloids, cardiac glycosides, carbohydrates,

steroids, phenolic compounds and tannins by using standard protocol [10].

Experimental Animals

The Swiss Albino mice (22-25g) of either sex were obtained from Sree Chithra Tirunal Institute of Medical Science and Technology, Poojapura, Thiruvananthapuram. Animals were housed in a group of four per cage and were maintained under natural day and night cycle at 25±2°C ambient temperature, 45-55% relative humidity. They were allowed to acclimatize one week before the experiment. The animals were allowed with free access to standard pellet and water *ad libitum*. The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and study protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Ref No: Sinchu Yesudanam /M.Pharm/KUHS/2014/a - d).

Acute Toxicity Study in Mice

Acute toxicity study was carried out on EEHV and AEHV following OECD guidelines (423)[11].

Experimental Design

Sedative & hypnotic activity

Phenobarbitone sodium induced sleep latency and sleeping time

Mice were divided into 5 groups containing 6 animals in each. On day of experiment, mice from all groups were placed in cages. The four test groups were administered with 200 and 400mg/kg of EEHV and AEHV. All the groups received phenobarbitone sodium 40 mg/kg (i.p) after thirty minutes of extracts to induce sleep. The animals was observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and the duration of sleep (time between the loss and recovery of righting reflex)[12].

Antidepressant activity

Forced swimming test (FST)

In a transparent glass cylinder (50 × 20 cm diameter) was filled with water up to 30 cm at room temperature. Animals were divided into 6 groups of 6 animals each, where first group was normal control. Second group was fluoxetine 10mg/kg (p.o) treated; groups 3-6 received 200,400mg/kg of EEHV and AEHV. Then, each animal was allowed to swim for 10 min during which immobility time was recorded. In total time period of 10 min, the duration of immobility was recorded during the last 6 min. After an initial 4 min period of vigorous activity, each animal assumed a typical immobile posture. Any reduction in immobility time was considered as antidepressant action[15].

Neurotransmitter estimation

After neuropharmacological screening, animals were used for the estimation of adrenaline and DA levels in brain. Mice were sacrificed and the brain was isolated out in an ice-cooled dissection slab, weighed and stored at -80°C for the monoamine analysis.

Anticonvulsant activity

Maximum Electric Shock (MES) Induced Seizure

Mice were divided into 6 groups containing 6 animals in each. On day of experiment, electrical shock was applied (120 mA for 0.2 s) to from group 2 to 6 through corneal electrodes to produce optic stimulation and cortical excitation, where first group was normal control. Second group was phenytoin 25mg/kg (i.p) treated, groups 3-6 received 200, 400mg/kg of EEHV and AEHV. The parameters like Flexion, extensor, clonus, stupor, recovery/death was observed [13].

Antianxiety activity

Open Field Test (OFT)

This open field behavioral assays are commonly used to test locomotor activity in rodents. The test area is made up of transparent walls and black floor (30×30×15cm) divided into 9 squares of equal areas. Mice were divided into 6 groups containing 6 animals in each in which, first group was normal control. Second group was diazepam 4mg/kg (i.p) treated, groups 3-6 received 200, 400mg/kg of EEHV and AEHV. The locomotor activity i.e the number of squares crossed was noted after 60min of drug administration at 0, 30, 60, 90 & 120min for a period of 3 min[14].

Briefly, the brain tissue was homogenized in 5ml acidified n-butanol and shaken for 5 sec. Then, the suspension was centrifuged at 3000xg for 5 min at 4°C. The supernatant was mixed with 2.5ml *n*-heptane and 0.31ml 0.1 M HCl, shaken for 10 sec and centrifuged at 3000xg for 5 min at 4°C. The aqueous phase was used for the further analysis.

To determine adrenaline and DA levels, 0.5 mL of the aqueous phase sample was pipetted into test tubes. 1.7 mL of phosphate buffer (pH7.2), 0.4mL of 0.1 M disodium ethylene diamine tetra acetic acid and 0.1 M iodine solution were added in order, then, let the tubes rest for 2 min after adding 0.5 mL of 0.2 M alkaline sodium sulfite, let them rest 2 min again. 0.5 mL of 6 M acetic acid was added at last. After heating in a boiling water bath for 2 min, tubes were cooled in water. To determine DA, samples were heated for 2 min and cooled again. Activation and fluorescent wavelengths of adrenaline & DA were at 440nm and 640 nm, respectively.

Concentrations of adrenaline & DA were calculated by compared with its respective standard substance. Obtained values were reported in ng/ml[16].

Histopathological studies

To know the extent of damage to brain, one part of the brain was conserved in 10% formalin and stained with hematoxylin and eosin and observed under light microscope.

High performance thin layer chromatography fingerprint study

Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) are methods commonly used for the identification and testing of raw materials and formulation regarding to their aspect of content uniformity, ensure purity, content stability and dissolution characteristics. HPTLC has been widely used for the phytochemical evaluation of the herbal drugs. Finger print analysis by HPTLC has become a valuable and important tool for concerning the chemical constituent in plants with botanical identity and also used for the evaluation of chemical and biochemical markers [17].

Preparation of sample solution

Accurately weighed 1g of *Humboldtia vahliana* Wight ethanol extracts were transferred to a 10 mL volumetric flask dissolved in 7 mL of ethanol. It was then sonicated for 10 minutes and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 10 mL with ethanol to get stock solution containing 100 µg in 1 µl.

Procedure

5µL of *Humboldtia vahliana* Wight ethanol extracts were spotted (total 8 spots) on TLC plate. The sample were spotted as sharp band of 6 mm width using spray on technique with a Camag 100 µL sample syringe on precoated silica gel aluminum plate $60F_{254}$, (10cm x 10cm) using a Camag Linomat V automatic sample applicator. The bands were applied at a distance of 10 mm from the bottom edge of the plate and the distance between the two bands was 11.4 mm.

Prior to chromatography procedure TLC plates were washed with ethanol and activated at 60°C for 5 min. Respective mobile phases(Toluene: Ethyl acetate: Formic acid(35:15:2)) were added in TLC twin trough developing chamber. TLC developing chambers were closed with lids and are allowed for saturation of solvent vapors. The sample loaded plates were kept in their particular mobile phase up to 70 mm. The developing chamber assembles were kept aside for saturation and development of chromatogram for 30 min at room temperature. After 30 min, developed plates were allowed to dry by hot air. The plates were photo-documented at UV 254 using photo documentation chamber. Finally, the plates were fixed in scanner stage and scanning was done at 200-400 nm. The plates were kept in photo documentation chamber and captured the images under, UV light at 254 and 366nm.

STATISTICAL ANALYSIS

Software instat was used for analysis of data , all values were expressed as mean±SEM using one way ANOVA followed by student Newman Keul's test. In case of brain neurotransmitter estimation, tests were performed in triplicate manner and the data were expressed as mean±SD. The criterion for statistical significance was set at $p < 0.05$.

RESULTS

The percentage yield with reference to crude plant material was calculated and is tabulated in table 1. The plant material of 700g was taken for each solvent for extraction. It was observed that the ethanol extract contains the highest percentage yield (%w/w) of 14.942.

Phytochemical screening of benzene, chloroform, ethanol and aqueous extracts of *H. vahliana* showed the presence of flavanoids, alkaloids, carbohydrates, phenolic compounds, tannins and absence of fixed oil , fats, saponins, proteins, amino acid, gum and mucilage, whereas steroids were found in benzene and chloroform extracts. The phytochemical screening of the extracts was tabulated in Table 2.

Prior to the neuropharmacological evaluation, acute toxicity study was carried out prior to know the therapeutic dose. EEHV and AEHV did not produce any toxic effect at the maximum dose of 2000 mg/kg body weight. Hence $1/10^{th}$ (200mg/kg) as lower dose and the next higher dose of $1/5^{th}$ (400mg/kg) was selected for the present investigation. The acute toxicity studies done on EEHV and AEHV were tabulated in Table 3.

The sleep latency and duration of sleep time after single oral administration of 200, 400mg/kg of EEHV and AEHV were shown in Table 4. It was evident that the mice which were treated with 400mg/kg of EEHV showed highly significant ($p<0.001$) effect on sleep latency and duration of sleep time than the lower dose (200mg/kg) of EEHV. Similarly 200 and 400mg/kg of AEHV also showed effect on sleep latency and duration of sleep time but which were comparatively lesser than that of EEHV, where 400mg/kg dose of AEHV showed the similar effect as that of 200mg/kg of EEHV. Overall the 400mg/kg of EEHV showed highly significant effect on this study.

In MES induced convulsion method, changed in duration of convulsion phase after oral administration of 200, 400mg/kg of EEHV and AEHV has been shown in Table 5. The mice which were treated with 400mg/kg of EEHV showed highly significant ($p<0.001$)

reduction in extensor phase of lower dose (200mg/kg) of aqueous extract was less significant ($p<0.05$) compared than other extracts .The two doses of AEHV and EEHV also reduced the duration of flexion, clonus, stupor and recovery phases of animals, whereas lower dose of AEHV showed less significant effect on all these phase of convulsion.

The influence of extract on locomotor activity in open field test after oral administration of two doses of EEHV and AEHV has been shown in Table 6. It was understood that the mice which were treated with 400mg/kg of EEHV showed highly significant($p<0.001$), the locomotor activity lowering effect (depressant effect) was evident at the 2nd observation period (30 min) and continued up to the 5th observation period (120 min) , than the lower dose (200mg/kg) of ethanol extract. Whereas higher and lower doses of AEHV showed better locomotor lowering effect at 120(Min) (23.54±0.65($P<0.001$) , 34.25±1.25($P<0.001$)). All the extracts significantly decreased the locomotor activity of the mice as the number of squares travelled by the mice at all doses of the extract was reduced significantly from the initial score. The results were comparable to those of the reference drug, diazepam.

The duration of immobility in forced swim test after oral administration of two doses of EEHV and AEHV has been shown in Table 7. Animals administered with Fluoxetine (10mg/kg)(p.o) showed the decreased duration of immobility, thus showing the significant ($p<0.001$) difference as compared to control. There was a significant ($p<0.001$) dose dependent decrease in duration of immobility in animals treated with 200 and 400 mg/kg doses of EEHV. It was noted that the mice which were treated with 400mg/kg of EEHV showed high significance ($p<0.001$) effect on duration of immobility due to the CNS depressant action than the lower dose (200mg/kg) of ethanol extract whereas, higher and lower doses of AEHV showed better effect as 199.51±0.65($P<0.001$) , 190.52±1.0.65($P<0.001$) respectively.

Phenobarbitone sodium induced sleep latency and sleeping time was monitored under different phases with respect to their brain neurotransmitter level, the various phases were distinguished with existing phase duration. The values of brain adrenaline and dopamine levels were tabulated in Table 8. Both the neurotransmitters levels ($4.53\times10^3\pm0.05$; $33.81\times10^3\pm0.32$) decreased in phenobarbitone sodium (40mg/kg)(i.p) treatment group. Single oral dose administration of two doses of adrenaline and dopamine reduced the neurotransmitter levels to ($2.96\times10^3\pm0.05$; $4.47\times10^3\pm0.04$) and ($20.01\times10^3\pm0.10$; $34.52\times10^3\pm0.10$) respectively in ethanol extract, similarly 400, 200mg/kg of aqueous extracts also reduced the level to ($5.71\times10^3\pm0.43$; $7.45\times10^3\pm0.51$ and $50.02\times10^3\pm0.10$; $53.51\times10^3\pm0.45$).

MES was given for animals to induce epilepsy and was monitored under different phases with respect to their brain neurotransmitter level, the various phases were distinguished with existing phase duration. The values of brain adrenaline and dopamine levels were tabulated in Table 9. Both the neurotransmitters levels ($2.48\times10^3\pm0.032$; $41.81\times10^3\pm0.26$) decreased in phenytoin (25mg/kg)(i.p) treatment group. Single oral dose administration of two doses of adrenaline and dopamine reduced the neurotransmitter levels to ($1.48\times10^3\pm0.32$; $2.95\times10^3\pm0.51$) and ($22.82\times10^3\pm0.26$; $30.71\times10^3\pm0.43$) respectively in ethanol extract, similarly 400, 200mg/kg of aqueous extracts also reduced the level to ($3.16\times10^3\pm0.15$; $4.13\times10^3\pm0.15$ and $33.83\times10^3\pm0.76$; $40.24\times10^3\pm0.26$).

The locomotor activity in OFT was monitored under different phases with respect to their brain neurotransmitter level, the various phases were distinguished with existing phase duration. The values of brain adrenaline and dopamine levels were tabulated in Table 10. Both the neurotransmitters levels ($5.01\times10^3\pm0.10$; $38.86\times10^3\pm0.32$) decreased in diazepam (4mg/kg)(i.p) treatment group. Single oral dose administration of two doses of adrenaline and dopamine reduced the neurotransmitter levels to ($3.01\times10^3\pm0.10$; $4.36\times10^3\pm0.15$) and ($15.33\times10^3\pm0.30$; $21.34\times10^3\pm0.60$) respectively in ethanol extract, similarly 400, 200mg/kg of aqueous extracts also reduced the level to ($5.02\times10^3\pm0.10$; $7.43\times10^3\pm0.10$ and $25.16\times10^3\pm0.76$; $34.03\times10^3\pm0.10$).

The duration of immobility in FST was monitored with respect to their brain neurotransmitter level, the various phases were distinguished with existing phase duration. The values of brain adrenaline and dopamine levels were tabulated in Table 11. Both the neurotransmitters levels ($6.53\times10^3\pm0.45$; $33.71\times10^3\pm0.20$) decreased in fluoxetine (10mg/kg) (p.o) treatment group. Single oral dose administration of two doses of adrenaline and dopamine reduced the neurotransmitter levels to ($2.46\times10^3\pm0.45$; $4.76\times10^3\pm0.32$) and ($24.41\times10^3\pm0.36$; $29.46\times10^3\pm0.25$) respectively in ethanol extract, similarly 400, 200mg/kg of aqueous extracts also reduced the level to ($5.56\times10^3\pm0.15$; $7.03\times10^3\pm0.40$ and $48.36\times10^3\pm01.01$; $51.71\times10^3\pm1.47$).

Histopathological studies in phenobarbitone sodium induced sleep latency and sleep time, MES induced convulsion, OFT and FST revealed that in general, the control groups showed white matter with thickened and dilated blood vessels, gliosis were seen. Microglia and apoptotic bodies are seen. Perking cells showed abundant cytoplasm. The standard groups showed proliferation of astrocytes. Astrocytes are placed closely, giving evidence of gliosis. Oligodendrocytes showed apoptosis. Ependymal lining appeared normal. No necrosis or granuloma seen. The

ethanol extracts revealed that white matter showed gliosis with thickened blood vessels. Apoptotic bodies are seen. Focal areas near glial cells showed hyaline eosinophilic bodies. Ependymal cells are reactive. Gray matter appeared normal. The aqueous extracts showed degenerative changes with vacuolization. Apoptosis were seen. Edema was moderately present. Blood vessels in white matter appeared histologically normal.

High performance thin layer chromatography fingerprint study

The HPTLC finger print study was performed on ethanol EEHV. The solvent system used was Toluene: Ethyl acetate: Formic acid (35:15:2).By performing TLC the mobile phase was identified after trial and error method by trying different mobile phases. Detection was performed under UV 254 nm and 366nm through which 6 peaks were observed. Ethanol extract of *Humboldtia vahliana* Wight showed there are six phytoconstituents and corresponding ascending order of Rf values started from 0.11 to 0.67 in which highest concentration of the phytoconstituents was found to be 41.22% and its corresponding Rf value was found to be 0.67 respectively and was recorded in Table12.

Table-1: Percentage yields of various extracts of Humboldtia vahliana Wight

PLANT NAME	PART USED	METHOD OF EXTRACTION	SOLVENT SYSTEM	PERCENTAGE YIELD(%W/W)
Humboldtia vahliana Wight	Bark	Soxhlet apparatus	Petroleum ether	0.154
			Benzene	0.571
			Chloroform	0.402
			Ethanol	14.942
			Water	5.54

Table-2: Phytochemical analysis of extracts of barks of Humboldtia *vahliana* Wight

Sl.No.	TEST	BEHV	CEHV	EEHV	AEHV
I	Alkaloids	+	+	+	+
II	Carbohydrates and Cardiac glycosides	+	+	+	+
III	Phytosterols	+	+	_	_
IV	Fixed oil and fats	-	-	-	-
V	Saponins	-	-	-	-
VI	Phenolic compounds and tannins	+	+	+	+
VII	Protein and Amino Acid	-	-	-	-
VIII	Gum and Mucilage	-	-	-	-
IX	Test for flavanoids	+	+	+	+

"+" Positive, "-" Negative

BEHV: Benzene extract of *Humboldtia vahliana* Wight, CEHV: Chloroform extract of *Humboldtia vahliana* Wight, EEHV: Ethanol extract of *Humboldtia vahliana* Wight, AEHV: Aqueous extract of *Humboldtia vahliana* Wight.

Table-3: Result of acute toxicity study at 2000mg/kg of EEHV and AEHV

SL.NO	PARAMETERS	RESULT
1	Motor activity	Absent
2	Tremors	Absent
3	Convulsion	Absent
4	Straub reaction	Absent
5	Pile erection	Absent
6	Loss of light reflex	Absent
7	Sedation	Absent
8	Muscle relaxation	Absent
9	Hypnosis	Absent
10	Analgesia	Absent
11	Ptosis	Absent
12	Lacrimation	Absent
13	Diarrhoea	Absent
14	Change in skin colour	No change

Table-4: Effect of EEHV and AEHV on phenobarbitone sodium induced sleep latency and sleeping time

SL.NO	TREATMENT GROUP	DURATION OF TIME(Min)	
		SLEEP LATENCY	SLEEP TIME
1.	Phenobarbitone sodium(40mg/kg)(i.p)	55±3.10	90.24±5.23
2.	EEHV(400mg/kg)(p.o)	14±1.42[a]	163.20±4.02[a]
3.	EEHV(200mg/kg)(p.o)	18.4±0.44[a]	142.34±3.30[a]
4.	AEHV(400mg/kg)(p.o)	19.23±1.11[a]	141.24±4.20[a]
5.	AEHV(200mg/kg)(p.o)	23.43±1.20[a]	97.30±6.04[a]

The data were expressed as M±SEM; (n=6).The data analysed by one way ANOVA followed by student Newman Kuel's test. a-p<0.001(***) compared to positive control.

Table-5: Effect of EEHV and AEHV on Maximum Electric Shock Induced Seizure

Sl.No	TREATMENT GROUP	DURATION OF TIME(Sec)				
		FLEXION	EXTENSOR	CLONUS	STUPOR	RECOVERY/DEATH
1.	Control	5.25±0.47	17±0.70	21.5±0.65	56±0.91	130±0.41
2.	Phenytoin(25mg/kg)(i.p)	1.50±0.28[a]	0.00±0.25[a]	14.25±0.48[a]	29.5±0.64[a]	82.5±0.96[a]
3.	EEHV(400mg/kg)(p.o)	1.50±0.28[a]	5.0±0.40[a]	13.25±0.48[a]	31.5±0.64[a]	96.0±0.71[a]
4.	EEHV(200mg/kg)(p.o)	3.0±0.40[b]	7.75±0.49[a]	16.75±0.48[a]	48.75±0.48[a]	112.25±0.85[a]
5.	AEHV(400mg/kg(p.o)	3.25±0.25[b]	10.0±0.41[a]	18.0±0.41[a]	52.5±0.65[b]	117.25±0.85[a]
6.	AEHV(200mg/kg)(p.o)	4±0.41[c]	15.25±0.48[c]	19.5±0.65[c]	54±0.41[c]	122.25±0.63[a]

The data were expressed as Mean±SEM; (n=6).The data analysed by one way ANOVA followed by student Newman Kuel's test. a-p<0.001(***) compared to positive control, b-p<0.01(**) compared to positive control. c-P<0.05(*) compared to positive control**.**

Table-6: Effect of EEHV and AEHV on locomotor activity in OFT

SL. NO	TREATMENT GROUP	DURATION OF TIME(Min)				
		0	30	60	90	120
1.	Control	115.25±0.48	104.35±1.34	94.0±1.08	86.15±0.65	74.25±1.04
2.	Diazepam(4mg/kg)(i.p)	94.25±1.11[a]	84.15±1.04[a]	53.25±1.49[a]	34.15±1.19[a]	24.0±2.42[a]
3.	EEHV(400mg/kg) (p.o)	45.0±1.29[a]	34.01±1.47[a]	25.0±1.29[a]	16.25±1.10[a]	3.50±0.65[a]
4.	EEHV(200mg/kg) (p.o)	65.25±1.49[a]	55.50±0.65[a]	34.25±1.93[a]	28.50±0.65[a]	19.51±0.65[a]
5.	AEHV(400mg/kg) (p.o)	71.25±1.10[a]	59.25±0.48[a]	40.45±0.65[a]	31.35±1.10[a]	23.54±0.65[a]
6.	AEHV(200mg/kg) (p.o)	88.50±0.65[a]	73.0±0.91[a]	54.0±1.47[a]	44.0±1.78[a]	34.25±1.25[a]

The data were expressed as Mean±SEM; (n=6).The data analysed by one way ANOVA followed by student Newman Kuel's test. A-p<0.001(***) compared to positive control**.**

Table-7: Effect of EEHV and AEHV on immobility in FST

SL.NO	TREATMENT GROUP	DURATION OF IMMOBILITY(Sec)
1.	Control	180.25±0.85
2.	Fluoxetine(10mg/kg)(p.o)	122.45±1.11[a]
3.	EEHV(400mg/kg)(p.o)	223.25±1.38[a]
4.	EEHV(200mg/kg)(p.o)	203.53±1.32[a]
5.	AEHV(400mg/kg)(p.o)	199.51±0.65[a]
6.	AEHV(200mg/kg)(p.o)	190.52±0.65[a]

The data were expressed as Mean±SEM; (n=6).The data analysed by one way ANOVA followed by student Newman Kuel's test. a-p<0.001(***) compared to positive control**.**

Table-8: Effect of EEHV and AEHV on adrenaline and dopamine estimation of Phenobarbitone sodium induced sleep latency and sleeping time

SL.NO	TREATMENT GROUP	NEUROTRANSMITTER LEVEL	
		ADRENALINE(ng/ml)	DOPAMINE(ng/ml)
1.	Phenobarbitone sodium(40mg/kg)(i.p)	$4.53\times10^{3}\pm0.05$	$33.81\times10^{3}\pm0.32$
2.	EEHV(400mg/kg)(p.o)	$2.96\times10^{3}\pm0.05$	$20.01\times10^{3}\pm0.10$
3.	EEHV(200mg/kg)(p.o)	$4.47\times10^{3}\pm0.04$	$34.52\times10^{3}\pm0.10$
4.	AEHV(400mg/kg)(p.o)	$5.71\times10^{3}\pm0.43$	$50.02\times10^{3}\pm0.10$
5.	AEHV(200mg/kg)(p.o)	$7.45\times10^{3}\pm0.51$	$53.51\times10^{3}\pm0.45$

All the extracts with respect to their concentration were tested in triplicate manner and the values are expresses as mean±SD

Table-9: Effect of EEHV and AEHV on adrenaline and dopamine estimation of Maximum Electric Shock Induced Seizure

SL.NO	TREATMENT GROUP	NEUROTRANSMITTER LEVEL	
		ADRENALINE(ng/ml)	DOPAMINE(ng/ml)
1	Control	$5.01\times10^{3}\pm0.10$	$50.06\times10^{3}\pm0.10$
2	Phenytoin(25mg/kg)(i.p)	$2.48\times10^{3}\pm0.32$	$41.81\times10^{3}\pm0.26$
3	EEHV(400mg/kg)(p.o)	$1.48\times10^{3}\pm0.32$	$22.82\times10^{3}\pm0.26$
4	EEHV(200mg/kg)(p.o)	$2.95\times10^{3}\pm0.51$	$30.71\times10^{3}\pm0.43$
5	AEHV(400mg/kg)(p.o)	$3.16\times10^{3}\pm0.15$	$33.83\times10^{3}\pm0.76$
6	AEHV(200mg/kg)(p.o)	$4.13\times10^{3}\pm0.15$	$40.240\times10^{3}\pm0.26$

All the extracts with respect to their concentration were tested in triplicate manner and the values are expresses as mean±SD.

Table-10: Effect of EEHV and AEHV on adrenaline and dopamine estimation of Open Field Test

SL.NO	TREATMENT GROUP	NEUROTRANSMITTER LEVEL	
		ADRENALINE(ng/ml)	DOPAMINE(ng/ml)
1	Control	$8.24\times10^{3}\pm0.26$	$44.81\times10^{3}\pm0.26$
2	Diazepam(4mg/kg)(i.p)	$5.01\times10^{3}\pm0.10$	$38.86\times10^{3}\pm0.32$
3	EEHV(400mg/kg)(p.o)	$3.01\times10^{3}\pm0.10$	$15.33\times10^{3}\pm0.30$
4	EEHV(200mg/kg)(p.o)	$4.36\times10^{3}\pm0.15$	$21.34\times10^{3}\pm0.60$
5	AEHV(400mg/kg)(p.o)	$5.02\times10^{3}\pm0.10$	$25.16\times10^{3}\pm0.76$
6	AEHV(200mg/kg)(p.o)	$7.43\times10^{3}\pm0.10$	$34.03\times10^{3}\pm0.10$

All the extracts with respect to their concentration were tested in triplicate manner and the values are expresses as mean±SD

Table-11: Effect of EEHV and AEHV on adrenaline and dopamine estimation of Forced swimming test

SL.NO	TREATMENT GROUP	NEUROTRANSMITTER LEVEL	
		ADRENALINE(ng/ml)	DOPAMINE(ng/ml)
1	Control	$8.41\times10^{3}\pm0.43$	$40.43\times10^{3}\pm0.30$
2	Fluoxetine(10mg/kg)(p.o)	$6.53\times10^{3}\pm0.45$	$33.71\times10^{3}\pm0.20$
3	EEHV(400mg/kg)(p.o)	$2.46\times10^{3}\pm0.45$	$24.41\times10^{3}\pm0.36$
4	EEHV(200mg/kg)(p.o)	$4.76\times10^{3}\pm0.32$	$29.46\times10^{3}\pm0.25$
5	AEHV(400mg/kg)(p.o)	$5.56\times10^{3}\pm0.15$	$48.36\times10^{3}\pm1.01$
6	AEHV(200mg/kg)(p.o)	$7.03\times10^{3}\pm0.41$	$51.71\times10^{3}\pm1.47$

All the extracts with respect to their concentration were tested in triplicate manner and the values are expresses as mean±SD.

Table-12: Depiction of peak table of EEHV

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	1	0.11 Rf	10.5 AU	0.14 Rf	107.5 AU	24.31 %	0.18 Rf	0.6 AU	2309.3 AU	11.99 %
1	2	0.24 Rf	7.3 AU	0.31 Rf	74.7 AU	16.90 %	0.38 Rf	0.1 AU	2992.5 AU	15.54 %
1	3	0.43 Rf	8.2 AU	0.51 Rf	24.2 AU	5.46 %	0.52 Rf	23.7 AU	863.6 AU	4.48 %
1	4	0.52 Rf	23.5 AU	0.56 Rf	31.4 AU	7.10 %	0.61 Rf	11.7 AU	1234.3 AU	6.41 %
1	5	0.61 Rf	12.1 AU	0.64 Rf	22.2 AU	5.01 %	0.67 Rf	10.3 AU	592.9 AU	3.08 %
1	6	0.67 Rf	10.5 AU	0.76 Rf	182.4 AU	41.22 %	0.91 Rf	1.9 AU	11270.2 AU	58.51 %

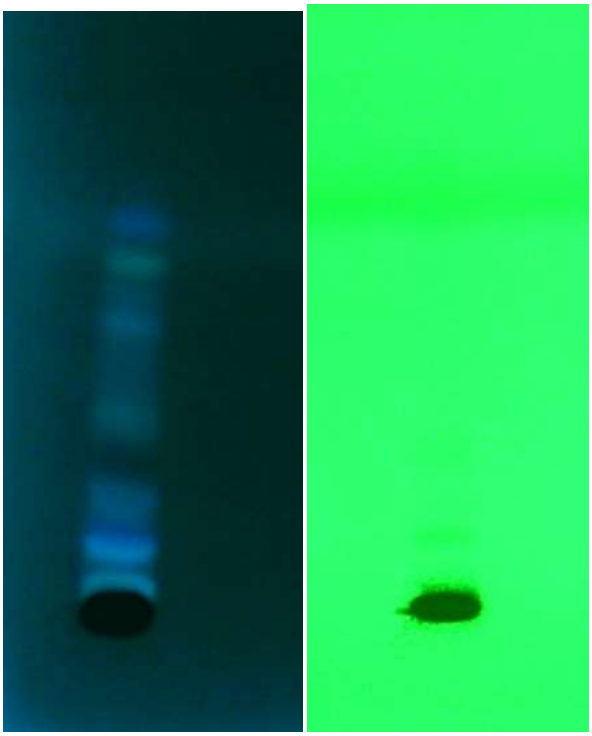


Fig-1: HPTLC plate seen at 366nm and 254nm

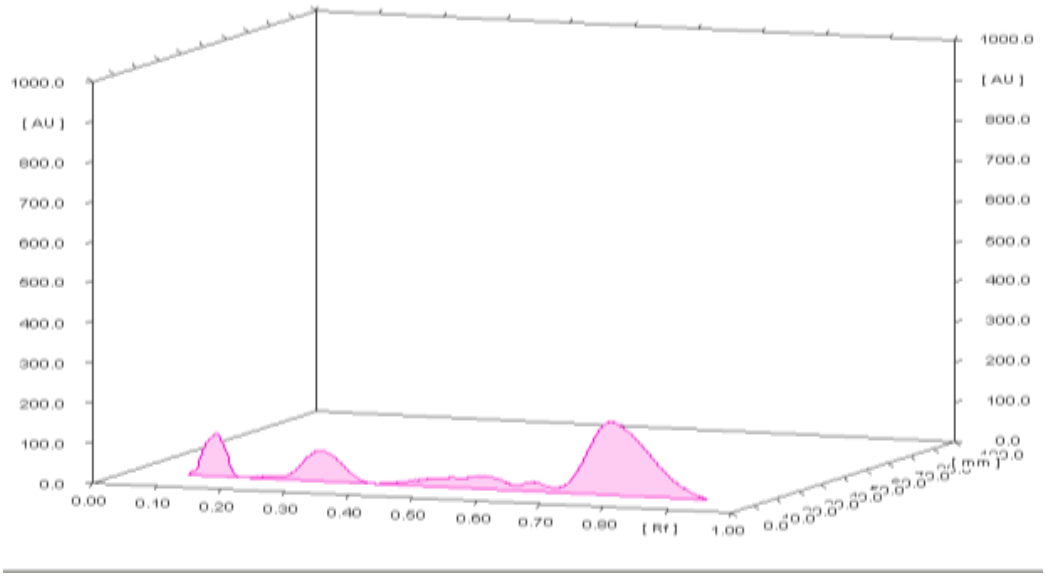


Fig-2: 3D display of EEHV

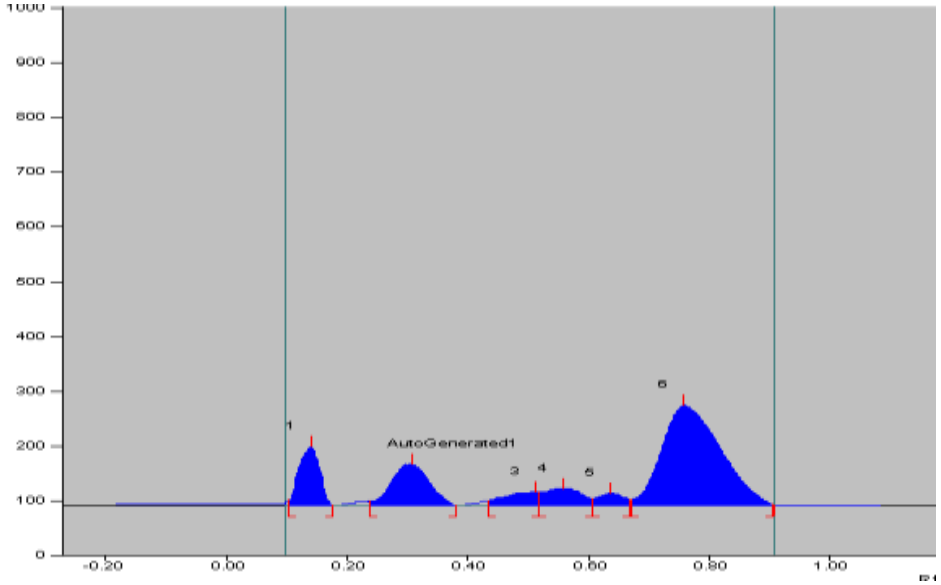


Fig-3: Peak display of EEHV

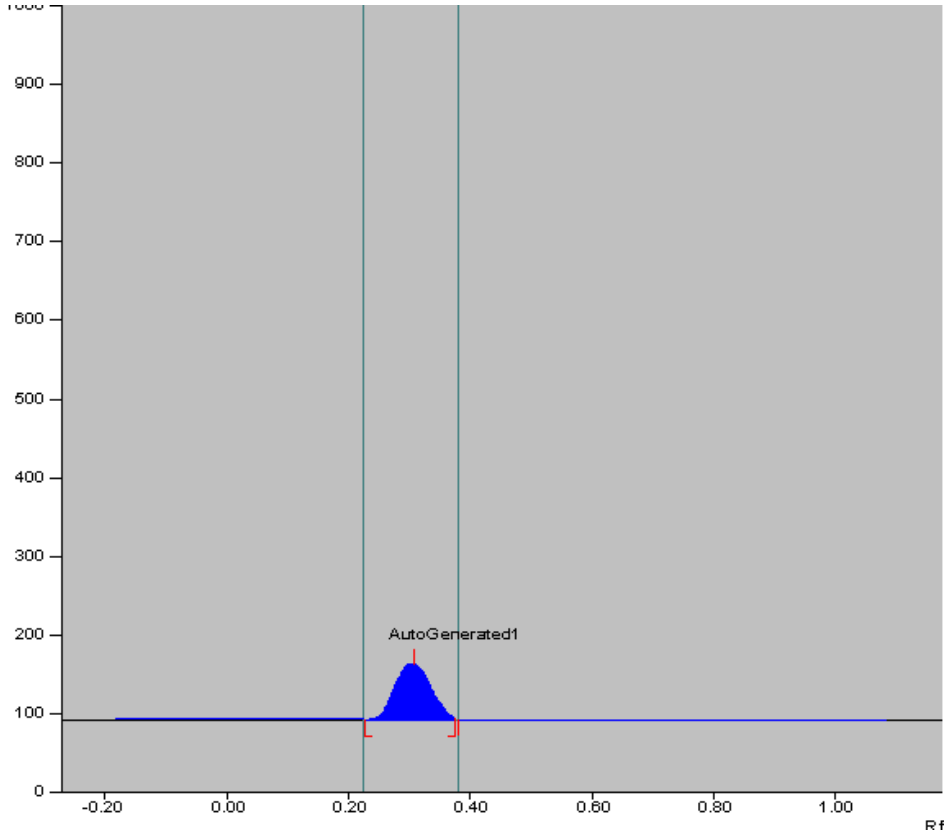


Fig-4: Peak display of the selected peak of EEHV

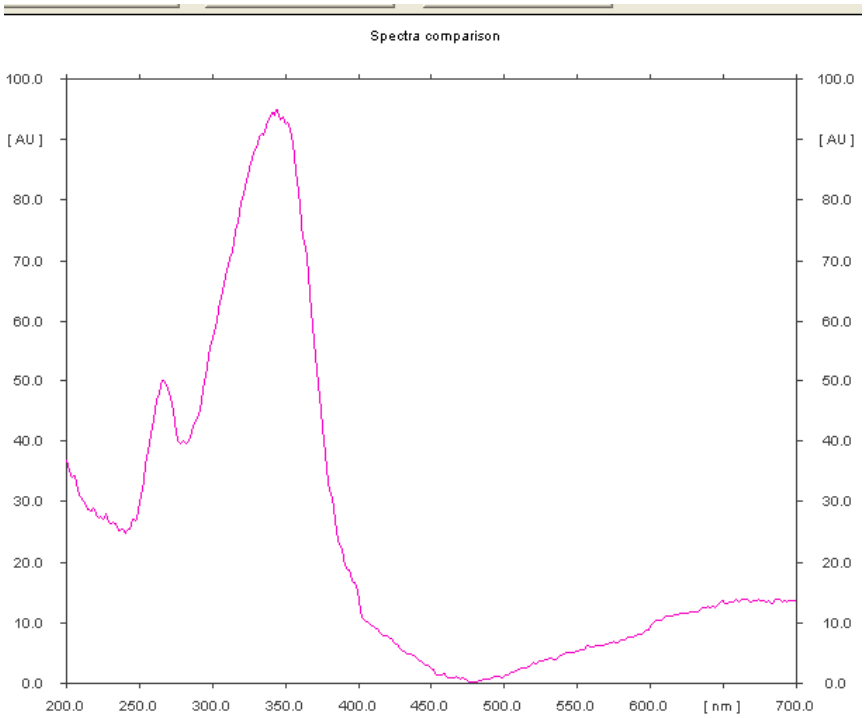


Fig-5: Spectral display of the selected peak in EEHV

DISCUSSION

The phytochemical screening of the bark extracts of *Humboldtia vahliana* Wight obtained from extraction with the aid of solvents like benzene, chloroform, ethanol and water, characterized the presence of flavonoids, alkaloids, carbohydrates, steroids, cardiac glycosides, phenolic compounds and tannins. The presence of flavonoids has been responsible for the neuropharmacological effect of the drug [18].

The results of acute toxicity study revealed that LD_{50} values of ethanol and aqueous extracts of *Humboldtia vahliana* Wight were high and apparently showed the safety of those extracts. The treatment of mice with ethanol and aqueous extracts of *Humboldtia vahliana* Wight *did* not change the autonomic or behavioural responses among mice. The zero percent mortality for ethanol and aqueous extracts of *Humboldtia vahliana* Wight *was* found at the doses of 2000mg/kg.

Barbiturates induce sleep by depressing the CNS. Thus, phenobarbitone sodium, a short to intermediate acting barbiturate produces quick onset of sleep as indicated by loss of righting reflex (inability to maintain posture) and the recovery was also detected as the animals regain their righting reflex [19].

The activity of EEHV and AEHV was assessed by recording mean latency time and duration of sleep by inducing phenobarbitone sodium. The study showed maximum reduction in sleep latency and increament in sleep time for higher doses of ethanol extract than the rest. This increase in duration of sleep after treatment corresponds the sedative activity or central depressant effects of the extracts due to stimulation of CNS inhibitory pathways [20]. Sedation occurs by potentiating GABA-induced chloride currents for prolonged periods [21].

It has often been stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na + channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-Daspartate (NMDA) receptor, such as felbamate.

EEHV and AEHV both doses used in the study has altered the flexion, extensor, clonic,stupor, recovery phases of MES seizures. Ethanol extract of Humboldtia *vahliana* Wight of higher dose was more effective in MES test. It may prolong the Na^+ channel inactivation and glutamatergic excitation through NMDA receptors than the other doses of extracts [13].

OFT are the most frequently used behavioural tests in pharmacology and neuroscience. Consequently, it has been used to study exploratory activity and anxiety-related behaviors in rodents. Open field behavioral assays are commonly used to test locomotor activity in rodents. The test area is made up of transparent walls and black floor(30×30×15cm) divided into 9 square of equal areas and the locomotor activity of the rodents are seen[14].

Open field test describes the locomotor activity (i.e) the number of square crossed) of mice in various doses of extracts. The depressing action of the extracts was evident and maximum for EEHV higher dose which shows much predominate CNS depressant effect.

It has been documented that the test drugs are quite sensitive to antidepressant drugs such as serotonin-specific reuptake inhibitors, tricyclics and MAO inhibitors. These antidepressant drugs remarkably decrease the duration of immobility in mice when they are allowed to swim in FST. The reduction in immobility time by the crude extract suggests that it may act through an interaction with the adrenergic or dopaminergic system. Moreover, the attenuation of oxidative stress by certain chemical constituents might also be responsible for the antidepressant action of crude extract.

In the conventional version of the animal model, an antidepressant effect is evaluated by a decrease in immobility during exposure to the un-escapable water tank [15]. The decrease in the immobility time is accompanied with the increase in swimming time .It was clarified that the EEHV(400mg/kg) showed a significant increase in immobility time and was highly significant($p<0.001$) which indicates CNS depressant effect.

Neurotransmitters are endogenous chemicals that transmit signals across a synapse from one neuron (nerve cell) to another "target" neuron. Neurotransmitters are stored in a synapse in synaptic vesicles, clustered beneath the membrane in the axon terminal located at the presynaptic side of the synapse. Neurotransmitters are released into and diffused across the synaptic cleft, where they bind to specific receptors in the membrane on the postsynaptic side of the synapse. A released neurotransmitter is typically available in the synaptic cleft for a short time before it is metabolized by enzymes, pulled back into the presynaptic neuron through reuptake, or bound to a postsynaptic receptor. Nevertheless, short-term exposure of the receptor to a neurotransmitter is typically sufficient for causing a postsynaptic response by way of synaptic transmission.

The role of neurotransmitters in sedative and hypnotics, phenobarbotone sodium promotes the binding on $GABA_A$ receptors thus potentiates GABA-induced chloride currents by prolonging period. It also can reduce glutamate-induced depolarizations of the AMPA subtype of glutamate receptor Thus, the activation of inhibitory $GABA_A$ receptors and inhibition of excitatory AMPA receptors by barbiturates may explain their CNS-depressant effects [22].

The role of neurotransmitters in epilepsy, phenytoin cause prolongation of the inactivated state of the Na^+ channel, facilitation of GABA mediated Cl^- opening and inhibition of T type calcium current. This probably contributes to their ant seizure action in the electroshock model and in partial seizures [23].

The role of neurotransmitters as anxiolytics, the drug diazepam have the capacity to promote the binding of the major inhibitory neurotransmitter GABA to the $GABA_A$ subtype of GABA receptors, which exist as multisubunit, ligand-gated chloride channels, thereby enhancing the GABA-induced ionic currents through these channels. Since receptor subunit composition appears to govern the interaction of various allosteric modulators with these channels, there has been a surge in efforts to find agents displaying different combinations of benzodiazepine like properties that may reflect selective actions on one or more subtypes of GABA receptors [22].

The role of neurotransmitters in antidepressants drugs like fluoxetine block the reuptake of serotonin, leading to increased concentrations of the neurotransmitter in the synaptic cleft and, ultimately, to greater postsynaptic neuronal activity [23].

The extracts significantly restored brain neurotransmitter levels (adrenaline and dopamine) and were estimated in phenobarbitone sodium induced sleep latency and sleep time, MES induced convulsion, OFT and FST. Thus the exracts protected the brain neurotransmitter levels and therefore the changes in behavior and parameter assessment were normal.

The HPTLC finger print study was performed on ethanol extracts of *Humboldtia vahliana* Wight. The solvent system used was Toluene: Ethyl acetate: Formic acid(35:15:2) and showed the presence of 6 peaks.

CONCLUSIONS

In the present study, the preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, carbohydrates, cardiac glycosides, steroids, tannins and phenolic compounds in the extracts of *Humboldtia vahliana* Wight barks which have been responsible for the neuropharmacological activity of the plant. Therefore, this plant merits further attention.

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