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Original Research Article

Evaluation of Pharmacological Activities of Fractionated Extracts of *Hoya parasitica* Wall. Leaves

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ABSTRACT

Hoya parasitica Wall. leaves are traditionally used to treat a number of ailments namely; rheumatism, kidney problems, jaundice, urinary tract disorder, constipation, fever, and pain. The purpose of the present study was to determine the phytochemical profile of n-hexane (NHPL) and ethyl acetate (EHPL) extracts of *Hoya parasitica* Wall leaves and to assess the neuropharmacological activities through elevated plus maze, open field, hole cross, and hole board tests together with cytotoxicity activity by brine shrimp lethality assay. In elevated plus maze test, significant time-dependent increase in anxiolytic effect was revealed by NHPL at a dose of 200 mg/kg whereas EHPL (200 mg/kg) showed significantly enhanced anxiolytic activity at 120 min compared to control ($p<0.05$). Moreover, in hole cross test, 200 mg/kg of NHPL demonstrated significant ($p<0.05$) dwindled locomotor activity during 5th observation period while time-dependent reduction of locomotor activity was exhibited by EHPL at a dose of 200 mg/kg. Again, in open field test, locomotor activity was found to be significantly dwindled by low dose of EHPL indicating sedative property whereas NHPL displayed significant sedative effect at the same dose at 30 min and 60 min compared to control ($p<0.05$). Head dipping activity was significantly ($p<0.05$) reduced in a dose-dependent manner by EHPL in hole board test which indicated CNS depressant effect. Furthermore, both extracts (200 mg/kg and 400 mg/kg) showed LC_{50} values of 172.874 µg/mL and 97.509 µg/mL, respectively. Therefore, the results of the present study disclose anxiolytic, sedative, CNS depressant and cytotoxic activities of *Hoya parasitica* Wall leaves.

***Keywords*:** *Hoya parasitica* Wall., Elevated Plus Maze, Open field, Hole cross, Hole board, Cytotoxicity.

Introduction

From ancient time, herbs and plant-based natural ingredients have been used as vital sources of foods and drugs.[1] Medicinal plants contain a variety of phytochemicals that are active against a number of diseases including cancer and psychiatric disorders.[2]Although synthetic chemicals are replacing the scarcity of natural sources, most of them produce severe unwanted effects. Natural sources are not only cheap but also rich in multiple phytochemicals having assorted pharmacological activities. In view of these perspectives, researchers are now-a-days turning their attention in search for new pharmacophore, with extended efficacy as well as least undesirable effects from natural sources.[3]

Depression is considered as one of the leading causes of long term disability among the psychiatric disorders across the world. At present, depression causes about 40.5% disabilities and over 20% of adults go through depressive episodes in their life. It has been predicted to be the primary cause of disability in the year 2030 by the World Health Organization Global Burden of Disease.[4] Success of present antidepressant and anxiolytic medications is questionable due to severe adverse effects like fainting, tremor, sexual dysfunction, CNS effects, urinary retention as well as physical dependency and tolerance.[5-7]

Moreover, oxidative stress-induced psychiatric disorders are less-responsive to usual anti-depressant and anxiolytic medications which act by either interaction with neurotransmitters such as nor adrenaline, serotonin, dopamine or alteration of synaptic transmission as well as neuromodulations.[8] About 70% of psychiatric patients do not get desirable effect using conventional antipsychotic medications while the remaining 30% are incapable of receiving current drug therapy due to high cost and some other reasons. Therefore, novel molecules having better anti-psychotic activity at lower cost along with reduced side effects and long term usability have been a great interest for researchers due to the demand of the current situation.[9]

Cancer is the second foremost cause of death in contemporary time. About 277 types of cancer are identified and their prevalence is increasing outrageously day by day.[10] Plant-derived current anti-cancer drugs may exaggerate the hope for searching new potential anticancer drugs with reduced side effects from natural sources.[11]According to National Cancer Institute (NCI, USA), brine shrimp bioassay is evidently correlated with *in vitro* growth inhibition of solid tumor cell lines in human since its utilization as a prescreening tool in the exploration of anticancer drugs.[12] *Hoya parasitica* Wall. Of Asclepiadaceae family, locally known as Cherapata, is an evergreen perennial shrub found in tropical wet forests and humid regions of southern Asia, Singapore, the Andaman Island, Australia and Polynesia. In Bangladesh the plant is grown in Chittagong and Chittagong Hill Tracts, the Sunderban, Sylhet, Moulvi Bazar, Cox's Bazar, Jashore, and Satkhira. It is a climbing epiphyte and charms parasites with fragrance of flowers.[11,13]A number of traditional uses of this plant have been reported in the treatment of rheumatism, kidney problems, jaundice, urinary tract disorder, bleeding disorder, paralysis,

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constipation, bronchitis, diabetes, fever, and pain.[14-18]Additionally, many researchers have reported antinociceptive, antioxidant, antimicrobial, hypoglycaemic, hypolipidaemic, antidiarrhoeal, thrombolytic, cholinesterase inhibition, cytotoxicity along with growth inhibitory effects.[11,19-22] Lupeol, lupenone, triterpenic 3,4-seco acid and 3,4-secolup-20(29)-en-3-oic acid, 15-bulnesolic acid, 1-(4-hydroxy-3-methoxyphenyl)-1 methoxypropan-2-ol, androstanoid, a sesquiterpene, a phenolics, triterpene and dihydrocanaric acid have been isolated from *H. parasitica*.[22,23]All evidences related to traditional uses may potentiate this plant as a significant source for new drug. Therefore, the present study was designed to conduct qualitative phytochemical screening along with neuropharmacological as well as pre-screening of cytotoxic activities of n-hexane and ethyl acetate extracts of *H. parasitica* Wall. leaves.

Materials and Methods

Chemicals and reagents
All reagents (analytical grade) procured from Sigma-Aldrich Co. (St Luis, MO, USA) were utilized in the present study. Moreover, two standard drugs (diazepam and vincristine sulfate) were purchased from Square Pharmaceuticals Ltd., Bangladesh and Beacon Pharmaceutical Ltd., Bangladesh, respectively.

Collection and identification of plant
H. parasitica Wall. leaves were collected from Sylhet, Bangladesh (24º53´11.1696´´ N and 91º52´50.5992´´ E), in January 2018. Expert of National Herbarium at Mirpur-1, Dhaka, Bangladesh named Sardar Nasir Uddin identified as well as authenticated the plant materials. A dried specimen with voucher number DACB-45169 was submitted to the Herbarium for future reference.

Preparation and of extraction of the plant material
Collected fresh leaves of *H. parasitica* Wall were washed with distilled water and shade-dried for 7 days. Then a laboratory grinding mill (MACSALAB 200 Cross Beated, Eriez®, Erie, Pennsylvania, USA) was used for grinding the plant materials into coarse powder followed by sieving through 40 meshes to get fine powder. For preparing n-hexane (NHPL) and ethyl acetate extracts of *H. parasitica* Wall leaves (EHPL), the fine pulverized powder (250 g for each solvent) was extracted with 1.5 L of n-hexane and ethyl acetate separately by hot extraction technique using Soxhlet apparatus. Then the extracts were concentrated by vacuum distillation at 68°C for NHPL and 76°C for EHPL in rotary evaporator (Rotavapor R-124, Buchi, Switzerland). The final yields of the extracts were 1.44% and 1.92% w/w for NHPL and EHPL, respectively.

Qualitative Phytochemical screening
Qualitative screening of a number of phytochemical groups such as alkaloids, glycosides, tannins, saponins, phenolics, flavonoids, carbohydrates, acidic compounds, steroids, gums, and anthraquinones was performed for both extracts.[12]

Collection and hatching of brine shrimp
The eggs of the brine shrimp (*Artemia salina*) were purchased from an aquarium shop (Jashore, Bangladesh) and mature shrimp called Nauplii were obtained after hatching the eggs in artificial seawater containing 3.8% NaCl for 48 h.

Experimental animals
Both male and female Swiss albino mice (50:50) having an average age and body weight of 6–7 weeks and 25–30 g, respectively, were obtained from Jahangirnagar University, Savar, Dhaka, Bangladesh. Adaptation of the animals to the experimental conditions was confirmed by keeping them in a standard housing condition comprised of relative humidity (55±5%), 12 h light/12 h dark cycle, and temperature (22±2℃) before 1 week of the commencement of experiment. All animals were subjected to overnight fasting condition prior to the experiments. The protocol designed for all experiments were approved by the Ethical Review Committee, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Bangladesh [Ref: ERC/FBS/JUST/2018-3(A)].

Experimental design
Each *in vivo* neuropharmacological study was accomplished with randomly selected 30 mice of either sex which were divided into six groups containing five mice in each. All groups were designed for oral administration of distinctive test materials as follows;
Group I (Control): Distilled water (10 mL/kg BW)
Group II: Diazepam (1 mg/kg BW)
Group III: NHPL (200 mg/kg BW)
Group IV: NHPL (400 mg/kg BW)
Group V: EHPL (200 mg/kg BW)
Group VI: EHPL (400 mg/kg BW)

Acute toxicity study
In acute toxicity study, LD_{50} (half lethal dose) was measured for experimental samples in accordance with the guidelines of OECD (Organization of Economic Cooperation and Development). Grouping of the mice was categorized as control and test groups (NHPL and EHPL, respectively) having five mice in each. Individual mouse of the respective group was subjected to oral administration of test samples at the doses of 100, 250, 500, 1000, 2000, 3000, and 4000 mg/kg body weight and observed for two weeks to monitor a variety of parameters including mortality, noisy breathing, diarrhea, injury, changes in locomotor activity, salivation, pain, discharge from eyes and ears, aggressiveness, food or water refusal, weakness, and coma for the confirmation of any sign of toxicity.[12,24]

Neuropharmacological study
Elevated Plus Maze Test
The method of Bakre *et al.*[25] and Moniruzzaman *et al.*[26] was followed to evaluate the anti-anxiety activity through a wooden elevated plus maze model having two open arms and two closed arms with the dimensions of 30×5×15 cm and 30×5×15 cm. The apparatus platformed on a wooden base was placed at a height of 38.5 cm from the floor. The apathy of the mice towards the open space as well as height was determined. Moreover, a trivial ridge of the open arms eradicated the slipping and dropping of the mice from the edge. After the oral treatment with distinct test material to the respective group, each mouse was kept at the maze center directing any of two closed arms. Then the number of entries in each type of arm was recorded at 0, 30, 60, 120, and 180 min for a period of 5 min. Entry was claimed only when all of four paws of mouse were into the arm. The apparatus was cleaned with 70% ethyl alcohol after testing each group.

Hole cross test
Hole cross test was performed using a specialized apparatus having cage-like shape (dimension: 30 cm × 20 cm × 14 cm) divided into two equal sized chambers connected by a 3.5 cm hole at the height of 7.5 cm in the dividing wall. Spontaneous movement of each mouse between two chambers through a hole was termed as number of passage and counted for 3 min at specified time intervals (0, 30, 60, 120, and 180 min, respectively) after oral administration of the specified test samples. Noise-free place and cleaning of the apparatus were maintained after testing each group.[26]

Open field test
The method of Aziz *et al.*[27] and Hafiz *et al.*[28] was followed to conduct open field test. In this method, an apparatus consisting of 76 × 76 square cm was divided into sixteen squares with alternative white and black color that contains a wall (40 cm high). In addition, the test board resembles a chess board. Each mouse of the distinct group was placed on the middle of the apparatus. Then the number of squares crossed was counted at specified time intervals (0, 30, 60, 120, and 180 min) after oral treatment of the test samples for a period of 3 min. The experiments were carried out in a calm and noise-free place and repeated for mice of each group.

Hole board test
The hole-board test was carried out utilizing the method of Bakre *et al.*[25] and Hafiz *et al.* [28] to evaluate sedative activity. The hole-board made of wood with a dimension of 40×40 cm contains 16 holes of 3 cm diameter and placed evenly on the floor. After 30 min of the oral administration of test samples, each mouse was kept at the centre of the board and the number of head dipping into the holes was recorded for 5 min. The experiment was carried out in a sound-free room and cleaning of the platform of the apparatus was done using 70% ethanol after each trial.

Cytotoxicity study
Brine shrimp lethality bioassay
The brine shrimp lethality bioassay was conducted to evaluate cytotoxic activity of NHPL and EHPL extracts following the method described by Sarkar *et al.*[12] NHPL and EHPL extracts were diluted serially with artificial simulated sea water and 20% pure dimethyl sulfoxide (DMSO) with the purpose of attaining desired concentrations ranging from 5 µg/mL to 320 µg/mL whereas 0.312 µg/mL to 5 µg/mL concentrations were prepared in the same manner for standard vincristine sulfate. Simulated sea water containing pure DMSO was used as control. Then sea water holding 10 live nauplii was taken in the test tubes containing prepared test sample of different concentrations and finally the volume was adjusted to 10 mL with sea water and kept for 24 h. Then LC_{50} was calculated by plotting the percentage of mortality of shrimp versus concentration of the test samples by using Ldp Line software.

Statistical analysis
Statistical analysis was performed with one-way ANOVA followed by Dunnett's test ($p< 0.05$; vs. control) and Post-hoc Bonferroni test ($p< 0.05$; vs. standard) using SPSS software (version20; IBM Corporation, New York, USA). Data were presented as mean ± standard error of mean (SEM) and considered significant at $p< 0.05$.

Results and Discussion

Qualitative phytochemical screening
Numerous phytochemicals were found to be present in preliminary phytochemical screening of NHPL and EHPL. Both extracts contained carbohydrates, alkaloids, phenolic compounds, flavonoids, tannins, glycosides, and gums whereas steroids were present in NHPL but not in EHPL. Saponins, anthraquinones, and acidic compounds were absent in both extracts. The results have been summarized in Table 1.

Acute toxicity study
Signs of toxicity or behavioral changes as well as mortality up to the highest dose of 4000 mg/kg body weight were not noticed throughout the observation period. Therefore, it can be claimed that, acute oral toxicity will be produced at the LD_{50} (half lethal dose) greater than 4000 mg/kg.

Neuropharmacological study
Elevated plus maze test
Elevated plus maze is a highly sensitive and validated method to define anxiolytic and anxiogenic behaviors expressed in terms of entry in open arms and closed arms, respectively. NHPL showed significant time-dependent increase in anxiolytic as well as decrease in anxiogenic effects compared to control at a dose of 200 mg/kg BW ($p< 0.05$). Moreover, reduction of anxiogenic effects by EHPL at a dose of 200 mg/kg was noticeable from 1st to last observation and significant effect ($p< 0.05$) was shown at 120 min. But higher doses of both extracts did not exhibit significant anxiogenic as well as anxiolytic effects (Figure 1).

Table 1: Qualitative phytochemical screening of NHPL and EHPL extract.

Phytochemical group	Test performed	Observation of NHPL	Observation of EHPL
Carbohydrate	Benedict's test	+	+
	Fehling's test	+	+
Alkaloid	Mayer's test	-	+
	Dragendroff's test	+	+
	Hager's test	+	+
	Wagner test	+	+
Phenolic compound	Ferric chloride test	+	+
	Dilute nitric oxide test	+	+
Flavonoids	Lead acetate test	+	+
	Alkaline reagent test	+	+
Tannins	Ferric chloride test	+	+
	Potassium dichromate test	+	+
Saponins	Froth test	-	-
Glycosides	Molisch's test	+	+
	Concentrated sulfuric acid test	+	+
Anthraquinone	Ammonium hydroxide test	-	-
Steroids	Salkowski's Test	+	-
Gums	Molisch's test	+	+
Acidic compounds	General test	-	-

Here, '+' indicates presence and '-' indicates absence

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Hole cross test

Hole cross test is an indicator of sedative as well as exploratory behavior expressed in terms of locomotor activity. From Figure 2, it was observed that, lower dose (200 mg/kg) of EHPL revealed significant decrease in the number of hole crossed in a time-dependent manner up to last observation as compared to control and standard ($p< 0.05$) whereas the same dose of NHPL showed significant dwindled locomotor activity ($p< 0.05$) at 180 min. Moreover, no significant activity was noticed by both extracts at 400 mg/kg.

Open field test

Open field test is used to evaluate sedative and exploratory behavior similarly to hole cross test. NHPL at 200 mg/kg exhibited significantly reduced locomotor activity during the second and third observation period (30 min and 60 min, respectively) compared to control ($p< 0.05$) while gradual decrease in locomotor activity was noticed from second observation period and continued up to the fifth period by EHPL (200 mg/kg) which was significant in comparison to control ($p< 0.05$) (Figure 3).

Hole board test

Hole board test is a well-established method to assess anxiolytic along with sedative-hypnotic activity in terms of exploratory behavior. EHPL showed a significant ($p< 0.05$) dose-dependent increase in sedative activity in relation to decreased number of head dipping compared to control and standard whereas a significant ($p< 0.05$) decreased number of head dipping was revealed by NHPL 200 mg/kg (Figure 4).

Cytotoxicity study

Brine shrimp lethality bioassay

There was an approximately linear correlation between a plot of percentage mortality and concentrations of the test samples as there was increased percentage mortality with the augmentation of the concentration in brine shrimp lethality bioassay. Cytotoxic activity of the experimental samples is defined in terms of LC_{50} (half lethal concentration) value. NHPL and EHPL revealed LC_{50} value of 172.874 µg/mL and 97.509 µg/mL, respectively in comparison to standard vincristine sulfate (0.577µg/mL) (Figure 5).

The presence of numerous bioactive compounds belonging to different phytochemical groups in plant extract is responsible for diverse activities of medicinal herbs. Some metabolites such as alkaloids, glycosides, phenolics, fatty acids, terpenoids, waxes, and their derivatives might possess medicinal properties. Hence, preliminary screening of secondary metabolites for the detection of bioactive compounds has been the prime concern for drug discovery as well as development.[12]

The acute toxicity study revealed that, oral administration of test samples at different doses (100, 250, 500, 1000, 2000, 3000, and 4000 mg/kg body weight) did not produce any sign of toxicity or behavioral changes as well as mortality even up to the highest dose of 4000 mg/kg body weight over the observation period. Therefore, it is obvious that NHPL and EHPL may not be toxic at the selected doses. However, there is a possibility of neurotoxicity at the higher doses of extracts in sub-chronic or chronic toxicity study.[25]

In the elevated plus maze test, exploration of animals into open arms is augmented rather than their aversion towards these arms. In contrast, the correlation of coerced or spontaneous movement of animals into the closed arms of EPM with their hormonal as well as behavioral changes implies elevated level of anxiety. Besides, fear and anxiety is indicated by the apathy towards the open arms. Thus, EPM test has been a reliable model to determine discerning anxiolytic activity of drugs.[29] Diazepam, buspirone, and other anxiolytics increase the time spent in open arm.[25]Among the different subtypes of $GABA_A$ receptor, there exists a multiplicity in their layout within 17 types such as α1 -6, β1 -3, γ1 -3, and others (single ε, θ, π and δ). Benzodiazepines exert their anxiolytic, sedative and amnesic property through binding with two subunits of $GABA_A$ receptor (α2 and α1, respectively). Moreover, Exiguous subunits of noradrenergic and/or serotonergic as well as other excitatory systems are responsible for anxiolytic effects of benzodiazepines.[30,31] Some researchers reported that, outright activation of glycine synapses in the brain may be involved in the anxiolytic action of benzodiazepines. Anxiolytic pharmacology may also be related to γ2 subunit of $GABA_A$ receptor.[29]

In EPM test, it was observed that, significant anxiolytic activity rather than anxiogenic effect was exerted by lower dose (200 mg/kg) of NHPL compared to control and standard ($p< 0.05$). Besides, significant reduction in anxiogenic effect was also exhibited by EHPL at the dose of 200 mg/kg compared to control ($p< 0.05$) at 120 min. So, this result suggests that both extracts revealed prominent anxiolytic activity which may be due to binding to α2 or γ2 subunits of $GABA_A$ receptor, the same mechanism through which benzodiazepines exert their action and may be by the activation of glycine synapses in the brain (Figure 1).

Both hole cross as well as open field tests were carried out to assess sedative property of NHPL and EHPL by observing locomotor activity of mice. In hole cross test, significant reduction in locomotor activity was shown by low dose (200 mg/kg) of EHPL in a time-dependent manner from initial to final observation period compared to control and standard ($p< 0.05$) while at same dose of NHPL it was noticed at 180 min (Figure 2). Besides, in open field test, dwindled locomotor activity was revealed by NHPL at 200 mg/kg during the second and third observation period compared to control ($p< 0.05$) whereas time-dependent reduction in locomotor activity from the second phase to final phase was revealed by EHPL 200 mg/kg compared to control ($p< 0.05$) (Figure 3).

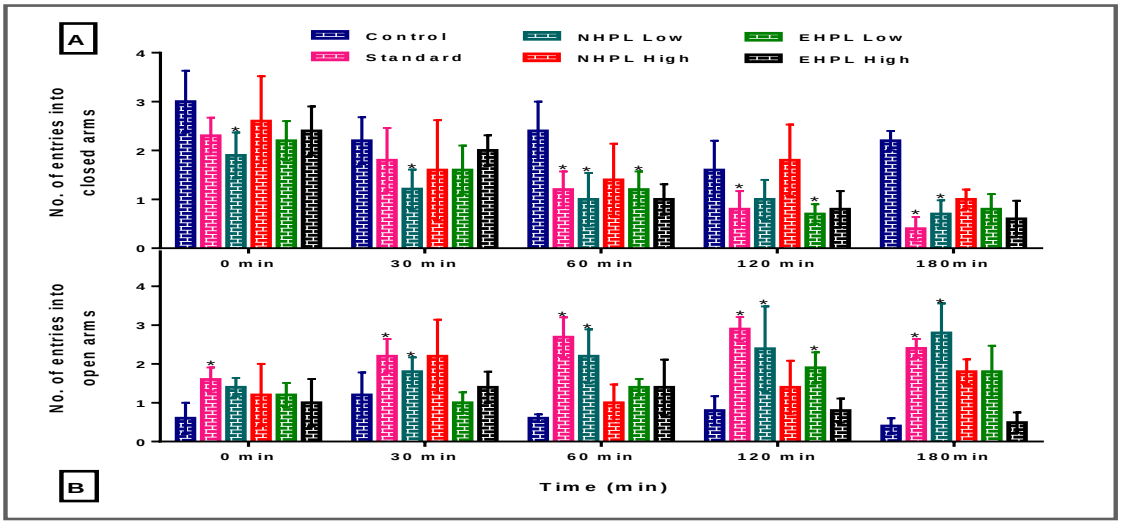

Figure 1: Effects of Diazepam (standard), NHPL and EHPL extract in elevated plus maze test

Values are expressed as mean ± standard error of mean (SEM), where n=5. *P<0.05, vs. control (distilled water, 10 mL/kg BW) (Dunnett's t test). $^{\theta}P$<0.05, vs. diazepam (standard) (post-hoc Bonferroni test). A and B represent the movement in closed arms and open arms, respectively. NHPL: n-hexane extract of *Hoya parasitica* Wall. leaves and EHPL: Ethyl acetate extract of *Hoya parasitica* Wall. leaves. Low and high indicate 200 and 400 mg/kg, respectively.

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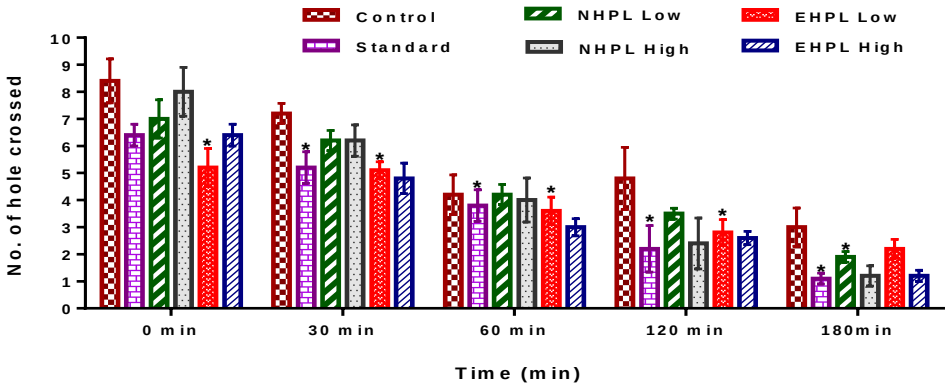

Figure 2: Effects of Diazepam (standard), NHPL and EHPL extract in hole cross test.
Values are represented as mean ± standard error of mean (SEM), where n=5. *P<0.05, vs. control (distilled water, 10 mL/kg BW) (Dunnett's t test). $^{\theta}P$<0.05, vs. diazepam (standard) (post-hoc Bonferroni test). NHPL: n-hexane extract of *Hoya parasitica* Wall. leaves and EHPL: Ethyl acetate extract of *Hoya parasitica* Wall. leaves. Low and high indicate 200 and 400 mg/kg, respectively.

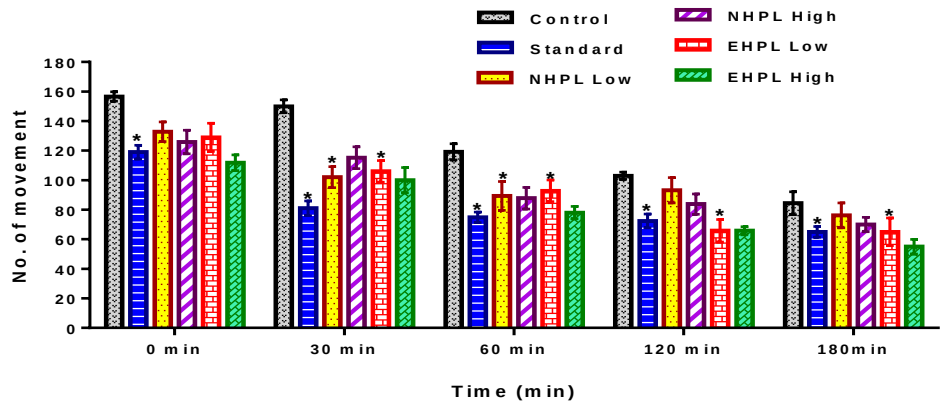

Figure 3: Effects of Diazepam (standard), NHPL and EHPL extract in open field test.
Values are presented as mean ± standard error of mean (SEM), where n=5. *P<0.05, vs. control (distilled water, 10 mL/kg BW) (Dunnett's t test). $^{\theta}P$<0.05, vs. diazepam (standard) (post-hoc Bonferroni test). NHPL: n-hexane extract of *Hoya parasitica* Wall. leaves and EHPL: Ethyl acetate extract of *Hoya parasitica* Wall. leaves. Low and high indicate 200 and 400 mg/kg, respectively.

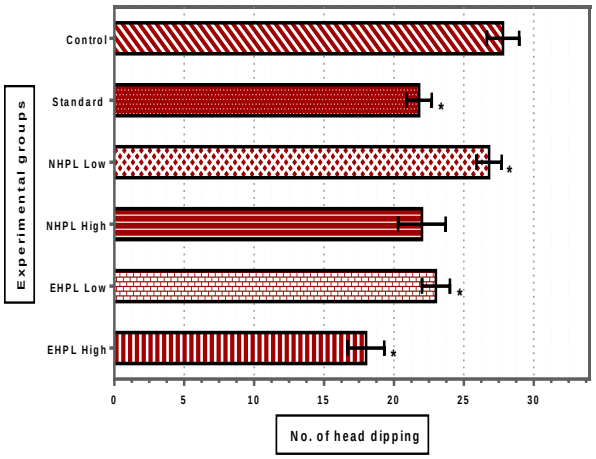

Figure 4: Effects of Diazepam (standard), NHPL and EHPL extract in hole board test.
Values are expressed as mean ± standard error of mean (SEM), where n=5. *P<0.05, vs. control (distilled water, 10 mL/kg BW) (Dunnett's t test). $^{\theta}P$<0.05, vs. diazepam (standard) (post-hoc Bonferroni test). NHPL: n-hexane extract of *Hoya parasitica* Wall. leaves and EHPL: Ethyl acetate extract of *Hoya parasitica* Wall. leaves. Low and high indicate 200 and 400 mg/kg, respectively.

So, the diminished locomotor activity by the plant extracts in our present study consolidated the CNS depressant effects. Significant suppression of locomotor activity was shown by both extracts in mice. Numerous psychological and neurological disorders related to physiological functions are linked with prime inhibitory neurotransmitter in the central nervous system called GABA (Gamma amino butyric acid).[32] The allosteric modification of GABA receptors is related to the potentiation of GABA-mediated postsynaptic inhibition by which assorted drugs might promote GABA system during it synthesis.[28,33] It causes suppression of voltage activated Ca^{2+} channel together with either augmentation of chloride conductance or promotion of GABA-induced chloride conductance.[34,35] Hence, it is extrapolated that, GABAergic inhibition in the CNS through membrane hyper polarization of critical neurons in the brain may be raised by these extracts. Also, GABA receptors may be incited by them. The affinity for GABA or the duration of the GABA-gated channel opening may be enhanced by these extracts.[36]

Hole board test is a validated method for the determination of anxiolytic along with sedative effects through the observation of exploratory behavior in rodents. It has been a convenient method due to some reasons like straightforward methodology, ease of observation of the behavioral responses of an animal and quantification despite exposure to a new environment. Moreover, a direct proportional relationship between the head-dipping behaviors of the animals and their emotional state was noticed.[26] On the basis of this observation, it was claimed that manifestation of anxiolytic state might be linked to

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the enhancement in head dipping behavior of the animal whereas dwindling in the number of head dips was found to be correlated with the depressant effect.[26,28] Our result showed that both doses of EHPL (200 and 400 mg/kg) and low (200 mg/kg) dose of NHPL revealed significant decrease in the number of head dipping as compared to control ($p< 0.05$) (Figure 4) indicating that both extracts possessed sedative activity rather than anxiolytic potentials.

Preliminary phytochemical screening of NHPL and EHPL revealed the presence of carbohydrates, alkaloids, phenolic compounds, flavonoids, tannins, glycosides, gums, and steroids (Table 1). Several reports have revealed that alkaloids, glycosides, and flavonoids enriched plant and plant extracts are accountable for sedative, anxiolytic, and antiepileptic properties due to their affinity for benzodiazepine site of GABAergic complex system leading to direct or indirect modulation of this receptor.[26] Besides, tannin has been enunciated for CNS depression potential through non-specific pathway.[28,37] It was also found that phytochemical groups like alkaloids, flavonoids, steroids, and terpenoids possess psychoactive effect because of the activation of protein kinase C (PKC) as well as transcription factors which are responsible for the multiple functions including initiation of the expression of cell-survival genes, conservation of neurons against a range of oxidative and metabolic insults, invigoration of nicotinic receptors leading to augmentation of cognition and memory, resuscitation and strengthening of the nervous function, activation of transient receptor potential calcium channels in the nerve cell membrane, etc.[38,39]

Brine shrimp lethality bioassay is a well-established *in-vitro* approach to analyze cytotoxic activities of medicinal plant extracts. Cytotoxic compounds are reported to show marked activity in this assay. There is a significant correlation between this bioassay and neoplastic cell lines of human. Positive result in this test has shown ovicidal and larvicidal effects.[11,40,41] Khatun et al. reported potential cytotoxic activity (LC_{50}~ 51.74 µg/mL) of methanol extract of *H. parasitica* stem.[11] Previous studies also have reported significant growth inhibitory activity against HeLa cells and SW480 cells by dihydrocanaric acid isolated from *H. parasitica*.[23]From the experiment it can be illustrated that the extracts may contain minor amount of cytotoxic compounds which are responsible for this cytotoxicity (Figures 5, 6 and 7).

Pharmacological activities of any plant are correlated to its secondary metabolites that are beneficial for plants and humans.[42] Therefore, the presence of several secondary metabolites in *H. parasitica* Wall. are correlated to the biological activities of the plant extracts and have been affirmed as a potential source of new drug molecules.

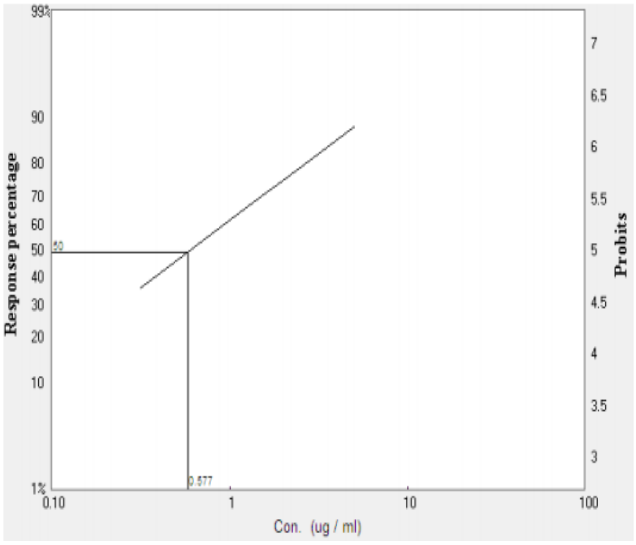

Figure 5: Graphical representation of brine shrimp lethality bioassay and LC_{50} for Vincristine sulfate

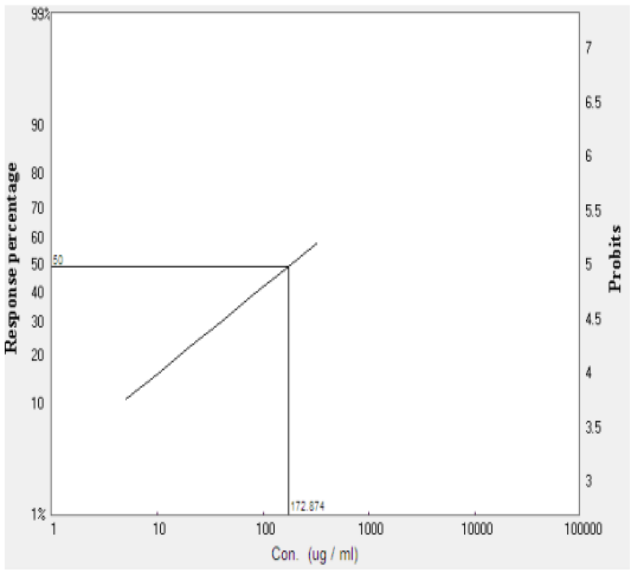

Figure 6: Graphical representation of brine shrimp lethality bioassay and LC_{50} for NHPL

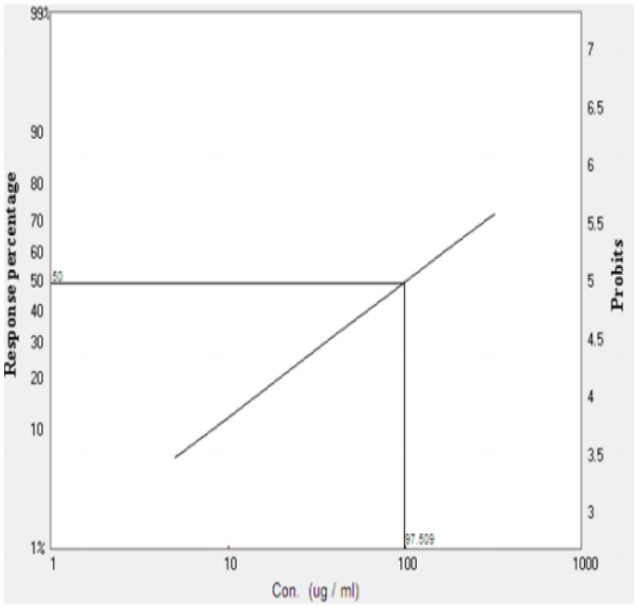

Figure 7: Graphical representation of brine shrimp lethality bioassay and LC_{50} for EHPL

Conclusion

From the present study it is evident that EHPL and NHPL have anxiolytic, sedative and CNS depressant properties through elevated plus maze, open field, hole board, and hole cross tests. Additionally, LC_{50} values of both extracts in cytotoxicity study were remarkable. Thus, it can be deduced that both NHPL and EHPL possess potential neuropharmacological as well as cytotoxic properties which rationalize the usage of *H. parasitica* Wall in folklore medicine. But advanced research is recommended to isolate new bioactive compounds as well as elucidate definite mechanism of action for these pharmacological activities.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the works presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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