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ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTI-PARKINSON ACTIVITY OF INDIAN MEDICINAL PLANT *HYDNOCARPUS PENTANDRA*

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ABSTRACT

Aim of the study: The aim of this research was to investigate the phytochemical profile, isolate the phytoconsitutuents, Characterised and evaluation of Anti Parkinson activity of ethanolic extract of Indian Medicinal plant of *H. Pentandra*. **Materials and methods:** Qualitative phytochemical analysis for their phytoconstituents. Ethanol was used to extract the crude bio active compound from whole *H. Pentandra* plant. Phenolic derivative were isolated from the ethanolic extract of *H. Pentandra* by using suitable solvent system and characterised by IR, NMR, MASS spectrophotometric method. The total Neuroprotective activity of *H. Pentandra* extract were measured by haloperidol induced experimental animal models. **Result:** Qualitative phytochemical analysis showed phenolic compounds, Flavonoids, Alkaloids, Terpenoids, Carbohydrates and Tannins were in high amounts. The isolated compound Phenolic derivative showed various region from IR, NMR and MASS the functional groups, mass of the molecule and proton were analysed and confirmed. *In-vivo* behavioral parameters like catalepsy, muscle rigidity and locomotor activity and the effects on neurochemical parameters in rats were studied using 200 and 400mg/kg, p.o doses of *H. Pentandra*. The increased haloperidol-induced cataleptic scores were significantly ($p<0.01$) found to be reduced, with EEHP at dose of 200mg/kg and 400mg/kg (ip). EEHP administration showed significant increase in dopamine level and significant reduced in serotonin and L-glutamate level. Daily administration of EEHP (400mg/kg) significantly improved motor performance. **Conclusion:** Overall results the study proved that *H. Pentandra* possessed potential components involving in anti -Parkinson treatment significantly attenuated the motor defects and also increased the neuro chemical dopamine level.

KEYWORDS

Hydnocarpus Pentandra, Anti-Parkinson's activity, Ethanol extract, L-Dopa, Carbidopa and Haloperidol.

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INTRODUCTON

Parkinson's disease (PD) is a neurodegenerative brain disorder characterized by progressive loss of dopaminergic and other neurons present in the substantia nigra pars compact a resulting in malfunctioning of the cerebral neuronal systems. Clinically the disease may cause slowness of movement, muscle rigidity, and rest tremor[1].

Pathologically, depletion of dopamine in brain due to the presence of intracytoplasmic inclusions known as Lewy bodies is caused. These pathological changes also observed in the locus coeruleus, pedunculopontine nucleus, raphe nucleus and dorsal motor nucleus of the vagal nerve[2] and sympathetic and parasympathetic postganglionic neurons. Drug management of Parkinson's includes drugs like levodopa, carbidopa, orphenadrine, benztropine, selegiline, pergola which reverses the symptoms of Parkinson's condition but with chronic use of these drugs various side effects like respiratory disturbances, hallucinations, salivary and urinary discoloration, dyskinesia convulsions and anxiety, arrhythmia, mydriasis, dry mouth, sore throat, and transient dizziness are observed[3].

Hydnocarpus Pentandra is an Indo-Malasian genus belonging to the family Flcourtiaceae. It is the most widely distributed species to Kerala and Tamilnadu. The stem, flower and leaves of *Hydnocarpus Pentandra* are used to scabies, leprosy, obesity, autoimmune disorders, wound healing, anti-inflammatory, antioxidant, rheumatism, sprains and chest infections. There have been very few scientific studies conducted on *Hydnocarpus Pentandra,* However, a comprehensive and exclusive study of the medicinal properties of *Hydnocarpus Pentandra*is still lacking. Hence, the present study aims to analyse the major phytoconstituent in ethanolic extract of the leaves and stems of *Hydnocarpus Pentandra.*

MATERIAL AND METHODS

Collection of Plant Material

Fresh leaves and stems of *Hydnocarpus Pentandra* were collected from natural locations in Kollihill, Tamilnadu and Andhra Pradesh region during July-September. This plant was identified and authenticated by Plant taxonomist, Department of Botony. Srivenkateswara University, Triupati. Voucher number 1176.

Animals

Wistar rats (150-200g) of either sex were obtained from Srinivasa Enterprises, Bengaluru, India, housed under standard light/dark cycle and fed with standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee, Chennai, India, approved (IAEC, P.Col/11/2017/IAEC/VMCP) experimental protocols and guidelines were followed in conducting the experiments on animals for the purpose of control and supervision of experiments on animals.

Preparation of Extract

Collected leaves were shade dried and coarsely grounded. 1000g of coarsely grounded powder material was weighed and extracted with 5liters of solvents like Petroleum ether (60-80^{o}C), Chloroform and ethanol by successive extraction method in a Soxhlet apparatus. After 72 hrs, the extraction solvent was distilled off and concentrated extract transferred into previously weighed petridish. Concentrate was evaporated to dryness at room temperature (45-50^{o}C) to obtain dried extracts. The extracts were weighed and the percentage yield of the extracts was calculated as follows:

$$\% \text{ of extractive yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried leaves powder}} \times 100$$

The yield of petroleum ether, chloroform and ethanol extract was 18.3, 11.5 and 26.6% (w/w), respectively.

Preliminary Phytochemical Studies

Qualitative chemical tests for establishing the chemical composition profile of given extracts were performed using standard methods/protocols[4] to detect various phyto constituents present.

Detection of Alkaloids

Alkaloids were detected by following in which solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. To few ml of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate formed indicates the test as positive.

Detection of Carbohydrates

The extract (100mg) was dissolved in 50ml of distilled water, filtered and subjected to the following test[5].

Fehling's test

One ml of filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicated the presence of sugar.

Benedicts's test

To 0.5ml of filtrate Benedicts reagent was added and heated on a boiling water bath for 2min. a characteristic red precipitate formed indicated the presence of sugar.

Detection of glycosides

50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2hr on a water bath, filtered and the hydrolysate was subjected to the following test.

Borntrager's test

2ml of filtrate hydrolysate and 3ml of chloroform was added and mixed together. Chloroform layer was separated and treated with 10% ammonia solution. Pink color indicated the presence of glycosides.

Detection of Saponins (Foam test)

50mg of the extract was diluted with distilled water and made up to 20ml.The suspension was placed in a graduated cylinder and shaken well for 15 min. Foam layer (2cm) indicated the presence of saponins.

Detection of proteins and amino acids

100 mg of the extract was dissolved in 10ml of distilled water and filtered through Whatman No.1 filter paper. The filtrate was then subjected to tests of proteins and amino acids.

Millon's test

To 2ml of filtrate, few drops of millon's reagent were added. A white precipitate indicated the presence of proteins.

Biuret test

An aliquot of 2ml of filtrated was treated with on drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by addition of excess of potassium hydroxide pellets. Pink coloration of the ethanolic layer indicated the presence of proteins.

Detection of Phenolic Compound

Ferric chloride test

50mg of extract in 5ml of distilled water was treated with few drops of 5% neutral ferric chloride solution. A dark green color formation indicated the presence of phenolic compounds.

Lead acetate test

50 mg of extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate was added. A dense white precipitate indicated the presence phenolic compounds.

Isolation of compound A

Preparation of Admixture of EEHP

15g of ethanolic extract of *Hydnocarpus Pentandra* was admixed with 20gm silica gel (60/120 meshes) to get uniform mixing.

Column packing for EEHP

200gms of silica gel was taken in a suitable column and packed very carefully without air bubbles using benzene as filling solvent and kept undisturbed for 1 hr for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by eluting with solvents of different polarity like benzene, chloroform, ethyl acetate and methanol. All the separated fractions were collected and concentrated under reduced pressure. Finally the column was flushed out using ethyl acetate and methanol.

Analysis of the isolated compound from the extract using various analytical techniques

Isolated compounds were characterized and identified by IR, NMR and GC-MS spectrophotometric methods and chemical structure of the compounds were subsequently elucidated.

Spectral analysis of the compounds using FT-IR

IR spectra of the compounds isolated from the extracts were recorded using Shimadzu. The spectral resolution for the Shimadzu was 0.25cm^{-1}, and the spectral data were stored in the database at intervals of 0.5cm^{-1} at 4000-2000cm^{-1}, and of 0.25cm^{-1} at 2000-400cm^{-1}. The samples were measured by using KBr disc method.

Spectral analysis of the compounds using ^{1}H NMR

^{1}H NMR spectra of the compound isolated from the extracts were recorded using a Bruker, USA (400 MHz). The measuring conditions for the most of the spectra were as follows: flip angle of 22.5-30.0 degrees, pulse repetition time of 30s. Repetition time of the longest pulse and small flip angle are

used to ensure precise relative intensities of the spectrum. The ^{1}H NMR chemical shifts were studied in reference to TMS and TSP in organic solvents and D_2O, respectively.

Spectral analysis of the compounds using ^{13}C NMR

^{13}C NMR spectra of the compounds isolated from the extracts were recorded with a Bruker, USA (400 MHz) spectrometer. The measuring conditions for the most of the spectra were as follows: a pulse flip angle of 22.5-45 degrees, a pulse repetition time of 4-7 seconds, and a resolution of 0.025-0.045 ppm. The resonance peaks present spectra were considered as Lorenz lines. The spectral codes in the spectra that resumes with CDS were modulated from peak positive, intensities and line widths. TMS (Tetra methyl silane) was used as the solvent for chemical shift.

Spectral analysis of the compounds using GC-MS

Mass spectra of the compounds isolated from the extracts were recorded with Shimadzu USA by the electron impact method where an electron accelerating voltage 75eV and an ion accelerating voltage of 8-10nV. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

Acute Oral Toxicity of the Extract

Randomised sampling procedure was adopted to carry out the acute toxicity study by using albino wistar rats. The minimum number of rats used for the study was 6. The animals of either sex were used as per OECD 423 guidelines. The animal were kept fasting for overnight providing only water, after which the extract was administered orally at the starting maximum dose level 2000mg/kg body weight by oral needle and observed for deaths of overt signs of toxicity ½, 1, 2 and 4 h after dosing and subsequently once daily for fourteen days. The signs of changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, motor activity and behaviour pattern were noted. The toxicity signs of fits, excessive salivation, tremors, diarrhea, lethargy, sleep and coma, as well as the onset of toxicity and signs of toxicity were also noted[6].

Haloperidol Induced Catalepsy (Model by Elliott and Close in 1990)[5]

Haloperidol causes dys functioning of various neurotransmitters such as acetyl choline, serotonin and GABA and induced catalepsy due to increased oxidative stress. Haloperidol, an antipsychotic drug, blocks central dopamine receptor in striatum and also produces a behavioral immobility and postural cataleptic state in animals characterized by muscle rigidity and failure to correct an externally imposed posture, thus haloperidol induced catalepsy model was selected. The method described by Elliott and Close in 1990 was followed for the anti-cataleptic activity.

Experimental Design

The animals were divided into five groups (n=6). Group I served as vehicle control, group II served as negative control, group III and IV served as test group treated with EEHP (200, 400mg/kg, i.p.) and group V served as standard L-DOPA + Carbidopa (100+25mg/kg, p.o.) respectively. Haloperidol (1mg/kg, i.p.) induced catalepsy was examined at every 30min interval for 180min using standard bar test. The duration for which the rat retains the forepaws extended and resting on the scale (block-method 0-3.5scale) was considered as cataleptic score.

Behavioral Assessment:

The different behavioral tests assessment were performed by blinded observer at different time points after lesion induction.

Locomotor Activity

The effect on locomotor activity was measured for 10 min at every 30 min upto 3 hours using actophotometer. The locomotor can be measured by using acetophotometer with photoelectric cells in circuit with a counter. When the beam of light falling on the photocell is cut off by animal, a count is recorded. Actophotometer has either circular or square arena in which animal movements are measured.

Exploratory behaviours

Head dipping[7]

The head dipping exploratory behaviour was measured by using the hole board, for 10minutes upto 3 hours at every 30minutes.

The plywood size of the hole board 3mm thick and 60cm X60cm. The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed holes each of 5 cm in diameter. The rat from each group was acclimatized and number of holes explored through head plunging acts for 10mintues. The total observation were noted. Care has to taken to avoid multiple events (two or more head plunging in quicker session). The animal plunged its head and did something else between grooming, short walk and etc, a fresh exploration was considered, before plunging its head for the next time. Each one animal from a group at a time was tested for each activity.

Line crossing[7]

The effect on exploratory behaviour (line crossing) was measured for 10 min at every 30 min up to 3 hours using by hole board. The hole board made of plywood of size (60cm X 60cm, 3mm thick). The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed lines. Each rat was acclimatized for 10 min and the number of line crossing acts, during the total observation period were counted. Care was taken to avoid multiple events.

Dissection and Homogenization

Rats brain was dissected out and subcortical region (including the striatum was separated. the Weighed quantity of tissue was homogenized in 3ml HCL-butanol in a cool environment. The sample was then centrifuged for 10 min at 2000rpm. The supernatant phase 0.8ml was removed and added to an reagent eppendorf tube containing 2ml of heptane and 0.25ml 0.1M HCL. After 10 min, shake the tube and centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for biochemical estimation.

Biochemical Estimation

Dopamine assay[8]

To 0.02ml of the HCL phase, 0.005 ml 0.4 ml HCL and 0.01ml EDTA/ Sodium Acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1ml sodium thiosulphate in 5M Sodium hydroxide. 10M Acetic acid was added 1.5 min later. The solution was then heated to 100ºC for 6 min. Excitation and emission spectra were determined using spectrofluorimeter at 330 - 375 nm for the samples at room temperature. Fluorescence (Test - Blank) values, with internal standard (0.005ml distilled water and 0.1ml HCL butanol to 20ng of dopamine standard), were measured.

SerotinineEstimation[8]

Serotonin assay

Tissue extract 0.5ml and 0.625ml of OPT (Orthopthaldialdehyde) reagent was heated to 100°C for ten min. After the samples reached equilibrium with the ambient temperature, excitation/emission spectra readings at 360-470 nm were taken.

Estimation of brain glutamate levels by UV assay method[9]

In this study animals were decapitated after the last behavioral session. The brains were immediately excised and cerebellum discarded. The cortex, striatum and the sub-cortical parts were separated and weighed. The sub-cortical region of the brain comprised all the remaining parts of the forebrain after dissection of the cerebral cortex and striatum, including the hippocampus, thalamus, hypothalamus, amygdale and other sub-thalamic structures. Spectrophotometer-suitable for precise measurement at 430 nm.

Deproteinization

Weighed quantity of brain portion were homogenized with 2 parts of perchloric acid by centrifugation for 10 min at 3000 rpm. Pippette out 3.0ml supernatant fluid and make up the pH to 9 with phosphate solution. Allow the solution to stand for 10 min in ice bath and filtered using fluted filter paper. Further, solution allowed to warm to room

temperature, diluted and take 1.0ml for the subsequent assays. Wavelength: 340nm, Light path: 1cm, Final volume: 3.35ml, Room temperature. For blank measurement, water used as blank instead of sample.

Pipette out 2.00ml of Glycine-hydrazine buffer, 1.00ml of Sample, 0.1ml of ADP solution, 0.2ml of NAD solution. Mix and read the extinction E_1 at 340nm. Then added 0.05ml of GIDH (Glutamate dehydrogenase) solution. Sample and Blank solutions were allowed to stand for 45 min and measured the extinction E_2 at 340 nm. Calculated the difference between E_1 and E_2 for sample and blank (ΔE). ΔE_{sample}- ΔE_{blank}= $\Delta E_{glutamate}$ is used for the calculations.

Statistical Analysis

The statistical analysis were carried out using analysis of variance ANOVA (between control and drug treatments), followed by Dunnett's 't' test. IP values <0.05 considered significant values and expressed as mean ± SEM.

Histopathological Studies

Histopathological study on the brains from control and experimental groups were done after fixing with 10% formalin, embedded in paraffin wax and sliced into longitudinal sections of 5µm. Further, Histopathology of these sections was observed using stained with hemotoxylin and eosin dye.

RESULTS AND DISCUSSION

Phytochemical Screening

Acute Toxicity

The EEHP dose of 2000mg/kg was found to be the maximal safe dose with no mortality. Therefore, lower dose of 200mg/kg and higher dose of 400mg/kg of EEHP were fixed as the study dose.

Effect of EEHP on haloperidol induced catalepsy (0-3.5 scale)

The cataleptic scores are depicted in Table No.1. There was a significant difference (P<0.01) between control group (I) and negative control group (II) in catalepsy. The EEHP treated groups shows significant anticataleptic action. EEHP at dose level of 400mg/kg particularly, shows anticataleptic action comparable to standard drug treatment. There was a significant difference (P<0.01) between negative control group (II) and EEHP in catalepsy. EEHP at dose level of 400mg/kg, showed good anticataleptic action at 30, 150, 180 min after haloperidol challenge.

Effect of EEHP on locomotor activity

The changes in locomotor activity after haloperidol administration are shown in Table No.2. There is a significant (P<0.01) decrease in locomotor activity in negative control group (II) when compared with the control group (I). EEHP 400mg/kg treated animals showed improved locomotor activity when compared with negative control group (P<0.01). EEHP at a dose of 400mg/kg potentiated the locomotor activity of standard drug. All groups were showing significant difference (P<0.01) when compared with negative control group at all time intervals.

Effect of EEHP on exploratory behaviour

The exploratory behaviour was expressed by head dippings and line crossings. Head dippings are shown in Table No.3. Negative control group (II) indicated decrease in exploratory behaviour i.e. head dippings and line crossings when compared with control group. The results presented by the EEHP treated groups show significant (P<0.01 and P<0.05) increase in head dippings and line crossings when compared with negative control group at 90, 120, 150, 180 min after haloperidol challenge.

Effect of EEHP dopamine levels

Dopamine levels are significantly (P<0.01) decreased in negative control group (II) when compared with control group. A significant (P<0.01) increase in dopamine value is observed in EEHP treated group animals. The results are showing increased dopamine levels in standard drug treated animals also. The results were shown in Table No.5.

Effect of EEHP serotonin levels

The present study shows significant (P<0.01) increase in serotonin levels in negative control group (II) animals. EEHP treated animals at both doses 200 mg/kg and 400 mg/kg showed significant (P<0.05 and P<0.01) reduction in serotonin levels when compared with negative control group. The results are shown in Table No.5.

Effect of EEHP on glutamate levels

The present study shows significant (P<0.01) increase in glutamate levels in negative control group animals. EEHP treated animals at both doses 200mg/kg and 400mg/kg showed significant (P<0.05 and P<0.01) decrease in glutamate level, which is a significant factor in excite toxicity. The results are shown in Table No.5.

Characterization of compound HP I

The isolated compound is dark yellow in colour, solid (128mg), soluble in methanol, ethanol and normal water and hot water. Melting point was found to be 134-142°C. The compound answered the test for carbohydrates. The mass of compound was found as 180 [M+1].

^{1}HNMR (Acetone - d_6, 400MHz) ppm

δ 7.75 (d, 2H, C_{1and4} - OH), 7.56 (dd, 2H, $C_{2\&3}$ - H),6.78 (m, 4H, $C_{7\&8}$ - H), 5.06 (s, 2H, C_5 - H).

^{13}CNMR: (Acetone-d_6, 400 MHz), ppm

δ 141C (C – 1 and 4), 124 C (C – 2 and 3), 116 C (C – 9 and 10), 99 C (C - 6), 80 C (C - 8), 79 C (C - 5).

Table showed the Phytochemical screening of the ethanolic extract of *H. Pentandra*

S.No	Compound	Result
1	Alkaloids	+
2	Glycosides	-
3	Flavonoids	+
4	Phenolic compounds and Tannins	+

Table No.1: Effect of EEHP on Catalepsy (0-3.5 scale)

S.No	Group	Catalepsy (0-3.5 scale)					
		30 min	60 min	90 min	120 min	150 min	180 min
1	I	0	0	0	0	0	0
2	II	2.90±0.13 ^{a**}	3.10±0.18 ^{a**}	3.40±0.15 ^{a**}	3.60±0.17 ^{a**}	3.60±0.15 ^{a**}	3.60±0.14 ^{a**}
3	III	1.29±0.12 ^{b**}	1.77±0.21 ^{b**}	2.09 ±0.17 ^{b**}	1.95±0.11 ^{b**}	1.47±0.17 ^{b**}	1.05±0.10^{b**}
4	IV	0.92±0.09 ^{b**}	1.70±0.10 ^{b**}	1.82±0.12 ^{b**}	1.61±0.11 ^{b**}	1.22±0.12 ^{b**}	0.70±0.09^{b**}
5	V	0.80±0.07 ^{b**}	1.05±0.10 ^{b**}	1.24±0.09 ^{b**}	1.05±0.05 ^{b**}	0.65±0.10 ^{b**}	0.42±0.12^{b**}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's 't' test. **P<0.01, *P<0.05, ns- Non significant.

Table No.2: Effect of EEHP on Locomotor activity

S.No	Group	Locomotor					
		30 min	60 min	90 min	120 min	150 min	180 min
1	I	321.52±7.24	329.22±7.14	325.19±7.62	337.19±5.33	331.28±6.17	334.33±5.29
2	II	29.19±2.17^{a**}	32.33±2.41^{a}	30.41±2.64^{a**}	31.22±1.92^{a**}	32.17±2.74^{a**}	34.28±2.52^{b**}
3	III	95.21±4.54^{b**}	169.24±5.28^{b*}	187.33±6.21^{b*}	205.69±6.33^{b*}	225.17±6.52^{b*}	244.67±7.54^{b*}
4	IV	110.28±5.27^{b**}	189.17±4.95^{b*}	205.33±5.21^{b*}	224.29±4.28^{b*}	251.33±4.87^{b*}	278.64±5.72^{b*}
5	V	128.05±6.27^{b**}	228.33±6.47^{b*}	244.17±6.54^{b*}	267.22±6.14^{b*}	272.19±5.21^{b*}	312.64±6.39^{b*}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's 't' test. **P<0.01, *P<0.05, ns- Non significant.

Table No.3: Effect of EEHP on Exploratory Behaviour - Head Dipping

S.No	Group	Head Dipping Behaviour					
		30 min	60 min	90 min	120 min	150 min	180 min
1	I	8.52±0.30	7.84±0.43	8.22±0.54	7.72±0.42	7.52±0.42	7.41±0.21
2	II	0.49±0.22^{a**}	0.45±0.31^{a**}	0.21±0.17^{a**}	0.52±0.22^{a**}	0.37±0.21^{a**}	0.59±0.22^{a**}
3	III	3.25±0.17 ^{b**}	5.17±0.31^{b**}	6.33±0.31^{b**}	6.95±0.26^{b**}	7.54±0.42^{b**}	7.69±0.31^{b**}
4	IV	4.65±0.52^{b**}	6.12±0.22^{b**}	7.56±0.37^{b**}	7.85±0.34^{b**}	8.14±0.46^{b**}	8.67±0.27^{b**}
5	V	5.21±0.22^{b**}	6.54±0.41^{b**}	7.69±0.33^{b**}	8.12±0.28^{b**}	8.29±0.51^{b**}	8.69±0.33^{b**}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's 't' test. **P<0.01, *P<0.05, ns- Non significant.

Table No.4: Effect of EEHP on Exploratory Behaviour - Line Crossing

S.No	Group	Line Crossing Behaviour					
		30 min	60 min	90 min	120 min	150 min	180 min
1	I	84.33±3.69	82.67±4.41	85.49±4.57	81.22±3.67	88.42±4.33	89.17±4.17 ^{a**}
2	II	5.29±0.54 ^{a**}	4.52±0.33 ^{a**}	4.19±0.41 ^{a**}	3.54±0.29 ^{a**}	3.22±0.57 ^{b**}	3.64±0.46 ^{b**}
3	III	18.24±1.17^{bns}	29.54±2.23 ^{b**}	35.22±3.62^{b**}	49.17±3.54 ^{b**}	60.21±4.33 ^{b**}	65.45±3.47 ^{b**}
4	IV	37.89±3.54 ^{b**}	57.67±4.33^{b**}	66.54±3.41^{b**}	72.19±5.12^{b**}	75.29±3.97 ^{b**}	76.42±3.28 ^{b**}
5	V	39.54±3.13 ^{b**}	59.22±4.80 ^{b**}	68.44±4.46 ^{b**}	74.19±4.75^{b**}	77.69±4.49 ^{b**}	79.54±5.28 ^{b**}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's 't' test. **P<0.01, *P<0.05, ns- Non significant.

Table No.5: Effect of EEHP on brain Neurotransmitter Levels in chronic haloperidol treated rats

S.No	Groups	Treatment	Dopamine (pg/mg tissue)	Serotonin (pg/mg tissue)	L-Glutamate (µ mol/gm tissue)
1	I	Control	754.69±12.47	274.33±5.17	74.19±3.22
2	II	Negative control (Haloperidol)	267.33±10.14 ^{a**}	412.27±4.67^{a**}	52.17±2.33^{a**}
3	III	EEHP (200 mg/kg)	554.42±8.12 ^{b**}	345.62±5.33^{b*}	59.21±2.37^{bns}
4	IV	EEHP(400 mg/kg)	617.28±10.44 ^{b**}	325.24±5.12 ^{b**}	64.33±3.11^{b**}
5	V	L-DOPA (100 mg/kg) + Carbidopa (25 mg/kg) as Standard	682.19±10.22 ^{b**}	307.58±4.69^{b**}	69.22±2.54^{b**}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's 't' test. **P<0.01, *P<0.05, ns- Non significant.

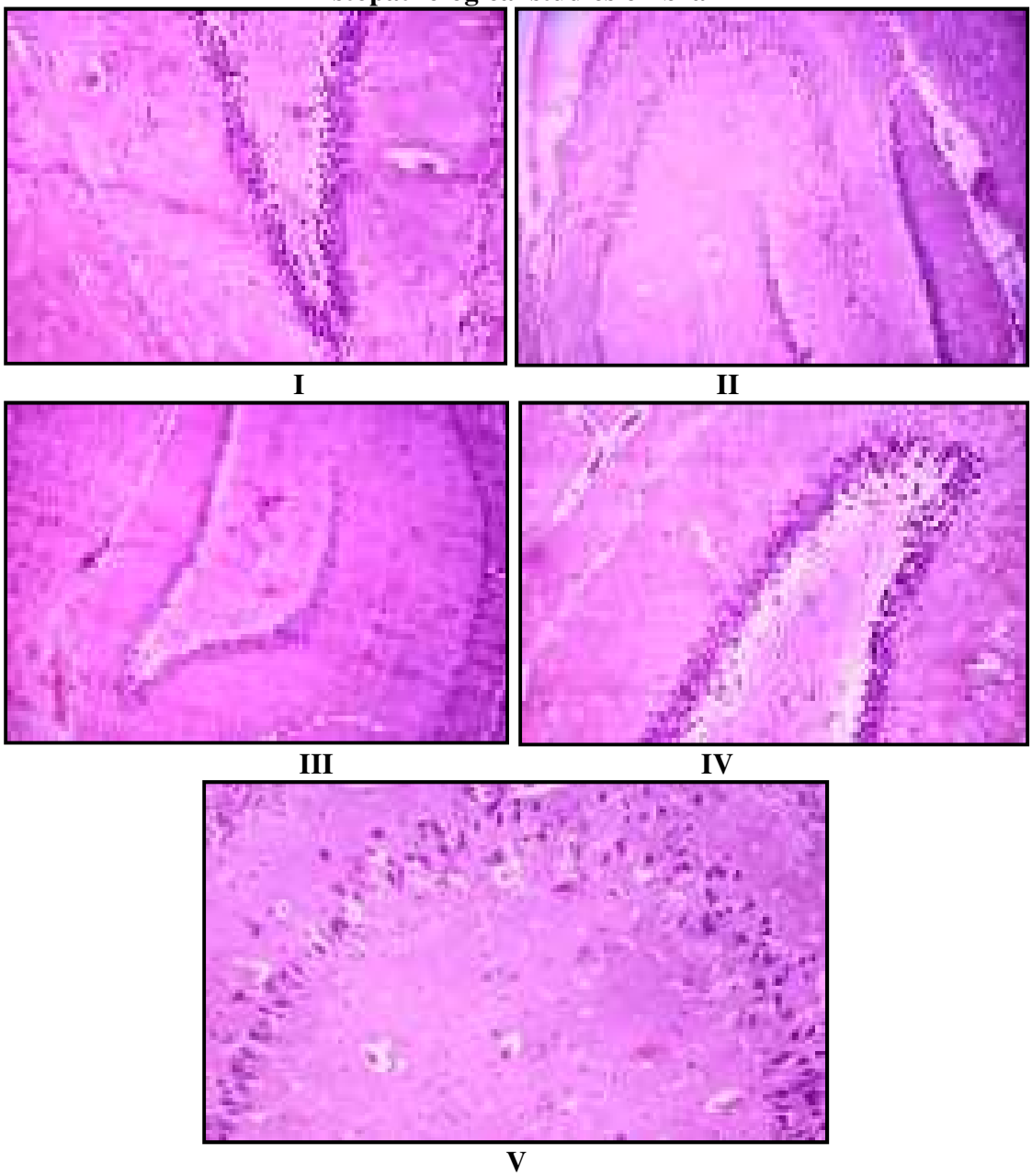
Table IR υ^{cm-1}: (KBr) Pressed pellet technique (HP I)

S.No	Wave numbers cm^{-1}	Type
1	3426	OH
2	2925	CH_3, CH_2, CH
3	2350	CH_2
4	1600	C=C
5	1371	C=C
6	1163	C-O-C

Table Properties of isolated compound HP I

S.No	Name	Compound A
1	Molecular formula	$C_{10}H_{12}O_3$
2	Melting point	134 - 142°C
3	Mass (m/z)	180 (M+1)
4	Colour	Dark yellow
5	Nature	Solid
6	Solubility	Soluble in methanol, ethanol and water
7	TLC studies	Chloroform: Methanol (R_f Value 0.33) 90: 10 (v/v)

Histopathological studies on brain



I **II** **III** **IV** **V**

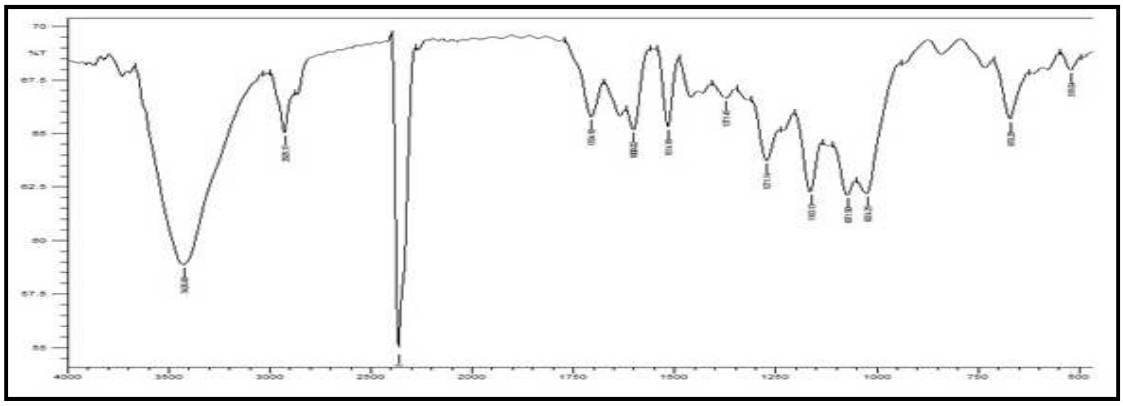


Figure No.1: IR Spectum of HP 1

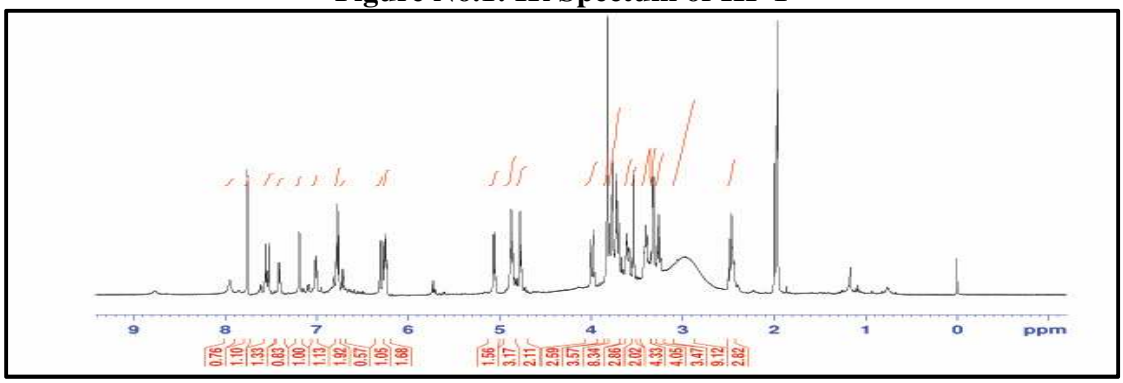


Figure No.2: HNMR Spectrum of HP 1

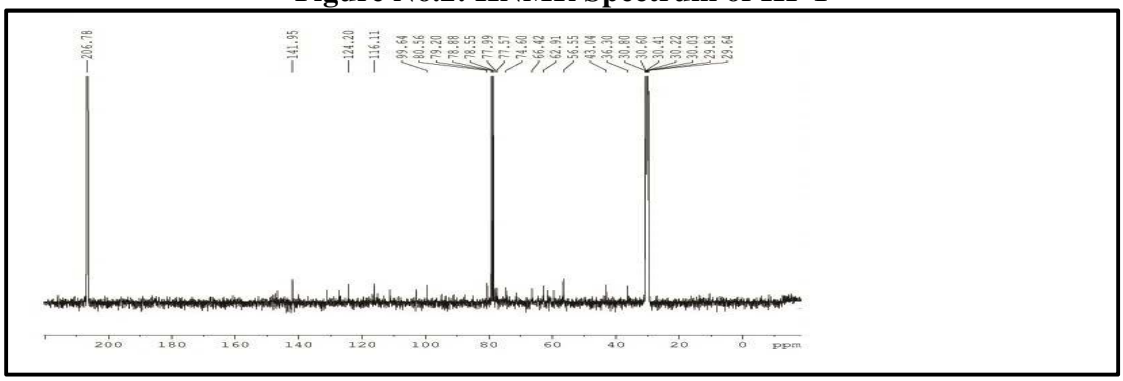


Figure No.3: CNMR of structure HP 1

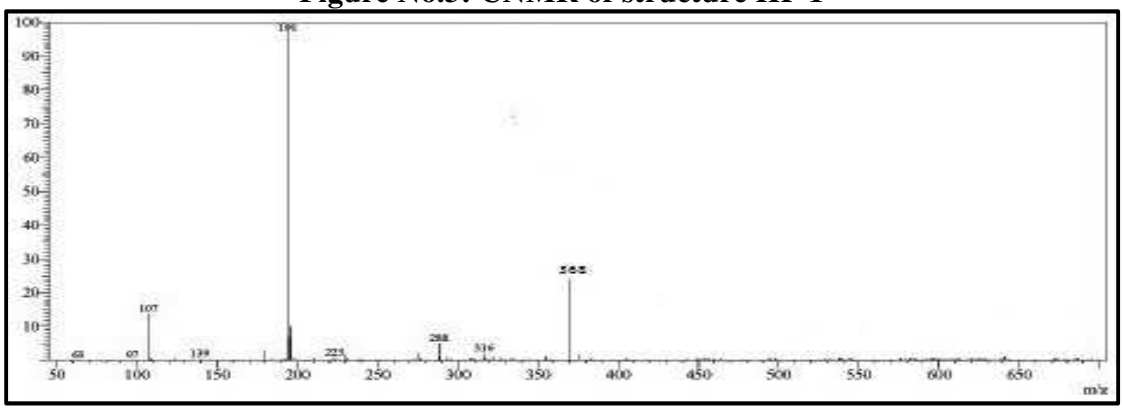


Figure No.4: MASS Spectrum of HP1

CONCLUSION

From these results of the present study, it was understood that *H. Pentandra* has higher anti Parkinson activity it may be understood from the variations in the profiles of the functional groups and phytochemical of the plants. In view of the above facts, we are concluding that ethanolic extract of *Hydnocarpus Pentandra* plant showed to a promising effect in animals with parkinson's disease. Further detailed molecular docking studies of the drug in anti-parkinson's evaluation pharmacology and toxicology as response for neuroprotective effect.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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