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Research Article

Evaluation of Antidepressant and Anxiolytic Activity of Fruit Extract of *Hylocereus undatus* in Experimental Animals

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ABSTRACT

Hylocereus undatus, generally known as dragon fruit was used in the present study to investigate antidepressant and anti-anxiety activity. In antidepressant activity we found that administration of 200 mg/kg and 400 mg/kg ethanolic fruit extract of *H. undatus* (EFEHU) and standard drug imipramine (20 mg/kg) for 21 days to respective group of animals they showed significantly reduced the immobility time in both tail suspension test (TST) and despair swim test (DST) in dose dependent manner as well as biochemical estimation was done. In anxiolytic activity administration of 200 mg/kg and 400 mg/kg EFEHU and standard drug diazepam (2 mg/kg) for 14 days to respective group of animals. In Elevated plus maze test (EPMT) significantly increased time spent in open arm and number of entries in open arm. The significantly increased level of 5-HT and GABA in brain of mice in dose dependent manner in both DST and EPMT. The phytochemical study of EFEHU showed the presence of alkaloids, tannins, carbohydrates, flavonoids, terpenoids, saponins, oils, phenolic compounds and qualitative examination by HPTLC technique showed that presence of quercetin and gallic acid. Therefore, the presence of such compounds in the extract may be responsible for the antidepressant and anxiolytic activity. Therefore, present study validates its antidepressant and anxiolytic activity. Further, research is required to elucidate its specific mechanism of action and isolation of responsible active principles.

Introduction

Depression and anxiety are the serious mental health conditions that impair the quality of life.[1] Different stress conditions arise during lifespan, and these circumstances may affect individuals differently, resulting in unspecific psychopathological manifestations such as depression and anxiety.[2]

Depression is a heterogeneous disorder which affects the mood, physical health, and behavior of an individual.[3] The symptoms ranging from very mild mood swings to extreme psychotic depression.[4]. The symptoms are depressed mood, diminished interest or pleasure, inability to concentrate or indecisiveness, significant rise or decline in weight or appetite, insomnia or hypersomnia, psychomotor agitation or retardation, feeling guilt, fatigue, and suicidal thoughts. These symptoms reflect change in psychomotor, cognitive, biological, motivational, behavioral and emotional processes.[5] The disorder is also often associated with suicide and there are 10–20 million suicide attempt each year.[6] Depression is effect on patient as well as family and friends also.

It is estimated that 7–12% of males and 20–25% of females experience a depressive episode in their lifetime. There are 2 types of depression, in which unipolar depression is first, where mood swings are in the same direction (continuously) and it is common (approximately 75% of case), non-familial, clearly connected with stressful life events. While the 2nd type is bipolar depression (approximately 25% of cases), sometimes it is also termed as endogenous depression, displays a familial pattern, not related to outside stresses and generally seems in early adult life, and is less common and it resulting in oscillating

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depression and also mania in excess of a few weeks.[5] There are symptoms in major depression that reflect variations in brain and in monoamine neurotransmitters (serotonin, nor epinephrine, dopamine).[7] Various evidence have supported that depression is caused by a permutation of genetic, biochemical, environmental and mental factors instead of a single cause.[5]

Anxiety is considered pathological or meaningless when it rises in the absence of challenge or stress, when it is beyond proportion with the challenge or stress in duration or severity, and when it results in psychological, biological social, occupational, and other impairment. Studies have reported high incidence of anxiety disorders in India with a majority among urban population and in females.[8]

A close connection has been accepted between anxiety and depression since ancient times. Thus, if anxiety is an integral aspect of depression using the treatment used in depression, anxiety may also be treated.[9] For treatment of depression reversible mono amine oxidase-A inhibitors, tricyclic antidepressants, SSRIs, SNRIs and atypical antidepressants are used which having a side effects like sedation, mental confusion, weakness, postural hypotension, sexual distress, gastrointestinal problems, etc. While anxiety is treated by drugs like SSRIs and benzodiazepines but this having adverse effects for example suicidal intention, decreased alertness, sexual distress, drug dependency and sleep disturbance, etc.[10] Now, researchers are looking for more specific drugs with higher safety and lower cost. The researchers working in this field have attention of medicinal plants because these plants have long been used to treat various diseases including psychiatric disorders with minimum adverse effects than synthetic and chemical drugs.[11] By screening natural sources like plant extracts, the search for novel chemical entities had led to the finding of various clinically beneficial drugs that play a vital role in the treatment of diseases. Currently, higher plants sustain to hold their historical status as significant sources of novel compounds that are useful either as medicinal agents (directly) or as lead compounds for synthetic/semi synthetic structural modifications/optimization or biochemical/ pharmacological probes.[5]

Hylocereus undatus having red - colored pericarp along with white flesh and it is native to Mexico and America.

The chemical constituents which are present in *H. undatus* are kaempferol, quercetin, b-sitosterol, isorhamnetin, gallic acid, betacyanin, alkaloids, terpenoids, steroids, tannins, glycoside, flavonoids, and phenolic compounds, saponins, proteins, amino acids, carbohydrate, vitamin B1, B2, B3, B6, vitamin C and minerals like calcium, copper, iron, phosphate and magnesium and this are useful for the antioxidant, anticancer property, anti-bacterial property, prebiotic effect, wound healing property, hypercholesteraemic effect, antiparkinson's and constipation activities.[12]

Need of Investigation

Depression and anxiety are the neuropsychiatric disorders. Mostly these occur as anxiety followed by depression or in mixed condition. Chronic use of available pharmacological agents (especially chemically synthesized) is related with serious adverse events. So, there is a necessity of alternate, effective, and safe ways to fight with various ailments. New era is moving towards using herbal compounds, nutrients, and supplements instead of rather than allopathic medicines as therapy options. What would be a better convenient treatment other than a conventional food.[13] Therefore, *H. undatus*, generally known as dragon fruit was used in the present study to investigate its antidepressant and antianxiety activity. It is a source of the natural antioxidant for prevention of degenerative disorders.

Promising anxiolytic and/or antidepressant effects have been reported from medicinal plants such as *Senna alata*,[4] *Withania somnifera*,[14] *Trema orientalis.*[15] It have been reported that the aerial parts hydroalcoholic extract of *Siphocampylus verticillatus* showed the antidepressant activity possibly due to the presence of alkaloid and other constituents including flavonoids, triterpenes and steroids.[16] Kailash Sharma *et al.*[17] in his article described that flavonoids (quercetin, kaempferol), triterpenoids (α, β- amyrin), sterol (β-sitosterol) as well as other phyto-constituents responsible for antianxiety activity.[17] It is reported that phytoconstituents present in the fruit of *H. undatus* are, flavonoids (quercetin, kaempferol), triterpenes (α, β-amyrin), steroids (β-sitosterol) etc.[12] Hence in present research work we select the *H. undatus* fruit to investigate antidepressant and antianxiety property for better efficacy, safety for long term use as well.

Materials and Method

Drugs and Chemical

Imipramine (Torrent Pharmaceutical Ltd. Ahmedabad), diazepam (Research-lab Fine Chem.-Industries, (Mumbai)), serotonin (Himedia, pvt Ltd.), gaba (Ozone international Mumbai), gallic acid (Research–lab fine chem industries. Mumbai), quercetin (Research Lab, Mumbai).

Equipments

High-performance thin-layer chromatography (HPTLC) system (AETRON), aluminium backed silica gel G_{60} F_{254} TLC plates (E-Merck), photofluorometer (Equiptronics), UV-visible spectrophotometer (Jasco V-730), centrifuge machine (Remi motors LTD), variable volume of micropipette (Biosystem), tissue homogenizer (Remi motors LTD), Shimadzu Instru, Mumbai (Analytical Weighing Balance), variable volume of micropipette (Biosystem).

Preparation of Extract

Fruit (*H. undatus*) were collected in month of September and October 2019 from Sangli region, Maharashtra, India.

The plant (*H. undatus*) material was authenticated by Prof. M. D. Wadmare, Department of Botany, Smt. Kasturba Walchand College Sangli, Maharashtra, India. Fruits of *H. undatus* were washed individually then separate out the pulp and peels grind the pulp and peel separately. Grinded materials were proceeding for drying. Dried material was powdered coarsely, mixed roughly and passed through sieve no. 40. Weighed quantity of powder with 95% ethanol was extracted using Soxhlet apparatus at 60–70°C boiling point of solvent then solvent was allowed to remove at 28–30°C (Fig. 1). The final extract was calculated using formula,

Percentage extract obtained = Wt. of extract (g)/Wt. of sample (g) ×100

Phytochemical Investigation

Phytochemical tests were carried out to find the presence of various phytoconstituents viz. carbohydrates, alkaloids, proteins, glycosides, flavonoids, steroids and tannins in fruit.[19]

Qualitative Estimation of Quercetin and Gallic Acid by HPTLC Technique

HPTLC of EFEHU with standard quercetin and standard gallic acid was done for qualitative identification.

Instrumentation

AETRON HPTLC system was used to analysis, which consist of,

- SPRAYLIN-V automatic TLC sample applicator
- CAMAG TLC Scanner-3 equipped with software Win CATS (version 1.4.2)
- Merck HPTLC plates coated with the silica gel G_{60} F_{254} (0.2 mm-thickness) on the aluminium sheets,
- ILS Micro syringe (100 µL)

Selection of Stationary Phase

Separation and the identification of all the drugs were performed on (10 cm x 10 cm, thickness layer 0.2 mm, E-Merck, Darmstadt, Germany) aluminium backed silica gel G_{60} F_{254} TLC plates.

Selection of Mobile Phase for Quercetin and Gallic Acid

Solvent system containing mobile phase – toluene: ethyl acetate: GAA: formic acid: methanol [5: 3:1:1:1 drop].

Preparations of Standard and Sample Solutions

Standard stock solutions of quercetin and gallic acid were prepared by dissolving 10 mg in 100 mL methanol to get stock solution containing 100 µg/mL of quercetin and gallic acid.

For preparation of sample solution- dissolving 10 mg of EFEHU in 10 mL methanol to get solution containing 1000 µg/mL.

Chromatographic Conditions

The experiment was performed on a silica gel G_{60} F_{254} (0.2 mm- thickness) HPTLC plates (10×10 cm) without prewashing. Samples were applied on the plates as 5 mm bands, 5 mm apart and 10 mm from the plate edges, with a SPRAYLIN-V automatic sample applicator. By using the ascending technique, the plates were developed to a 80 mm distance, at 25 ± 5°, relative humidity (50–60%), in a Camag twin-trough glass chamber (with a stainless steel lid), by mobile phase, which composed of toluene: ethyl acetate: GAA: formic acid: methanol [5: 3:1:1:1 drop] for quercetin and gallic acid. The saturation time for chamber was kept as 10 minutes. Later development, plates were dried, observed in a Camag UV cabinet, then scanned through Camag TLC Scanner, using software win CATS (version 1.4.2), in absorbance mode (with slit dimensions 6.00 X 0.45 mm), micro, whereas wavelength 259 nm was selected.

Animals

All experiments were done using male and female, Swiss Albino mice. Young (6–8 weeks) mice weighing around 18–24 gm were procured from animal house of Appasaheb Birnale College of Pharmacy, Sangli and were used for study. Form B protocol were prepared and submitted to Institutional Animal Ethics Committee (AIEC). The animal had free access of food and water and they were kept in natural light-dark cycle. To the laboratory condition the animals were acclimatized for as a minimum 5 days before conduct experimentation. The protocol of experiment was approved (IAEC/ABCP/092019-20) by the IAEC (Institutional Animal Ethics Committee) and laboratory animals care was taken according to guidelines of CPCSEA.

Dose Selection [12]

The doses were selected which depends on the acute toxicity studies from the literature survey. So, the doses selected $1/10^{th}$ and $1/5^{th}$ of 2000 mg/kg is 200 mg/kg and 400 mg/kg.

Experimental Design [18,20,21]

The animals were divided into 4 groups (6 mice in, respectively group). All animals were administered the drugs/extract (according to treatment) orally by using tuberculin syringe (1-mL) and gavage needle.

Antidepressant Activity

The duration of treatment was given for 21 days. On 7^{th}, 14^{th} and 21^{st} day all groups were evaluated for antidepressant activity through TST and FST method and on 21^{st} day animal was sacrificed and further biochemical tests were performed.

Tail Suspension Test (TST)

Group I: Control-distilled water (10 mL/kg p.o.)
Group II: Standard-imipramine (20 mg/kg p.o.)

Group III: Test I-EFEHU (200 mg/kg p.o.)
Group IV: Test II- EFEHU (400 mg/kg p.o.)
In TST model, takes immobility as a parameter of helplessness, which is proved by zero body movement of animal (or passive hanging) by using adhesive tape, mice were hung the upside down by their tail, at 58 cm (height from the ground), on the table side, for 6 minutes. Documented the length of the immobility for 6 minutes, without the early struggling 2 minutes.

Despair swim test (DST)

Group I: Control-distilled water (10 mL/kg p.o.)
Group II: Standard-imipramine (20 mg/kg p.o.)
Group III: Test I-EFEHU (200 mg/kg p.o.)
Group IV: Test II-EFEHU (400 mg/kg p.o.)
In this model, mice were forced into restricted area to swim. It produces a mental state of hopelessness (despair behaviour) in rodents, that was accounted for in the state of immobility period, which resembles to depressed state of human. Mouse was forced to swim individually in a plastic cylinder having dimensions (height: 40 cm; diameter: 18 cm) and containing 15 cm height fresh water and maintained at 25°C (± 30c). Animal was thrown down in water after 1-hour of treatment and for 6 minutes its activity was documented. The initial vigorous 2 minutes activity is its escape-oriented action. The phase of activity was slowly to swimming inactively or floating just to hold head above the level of water, reflected its behaviour of despair. No reading was taken in the first 2 minutes, and behavior of despair was recorded in the next 4 minutes.

Anxiolytic Activity

Elevated Plus Maze (EPM) Apparatus

The duration of treatment was given for 14 days. On 7^{th} and 14^{th} day all groups will be evaluated for anxiolytic activity by EPM apparatus. On 14^{th} days animal were sacrifices and further biochemical tests were performed.
Group I: Control-distilled water (10 mL/kg p.o.)
Group II: Standard-diazepam (2 mg/kg I.p.)
Group III: Test I-EFEHU (200 mg/kg p.o.)
Group IV: Test II-EFEHU (400 mg/kg p.o.)
The maze was plus-shaped (with an open roof), containing of 2 open arms (16, 5 cm), also two enclosed arms 16, 5, 12 cm, and 25 cm height. Each mouse was alone positioned in the center of EPM (with its head in front of an open arm) then observed for 5 minutes to record the no. of open arm entries, closed arm and time spent in each arm. Only when overall 4 paws were in the respective arm then mouse was measured to have entered or spent time in a respective arm. After individually test, the maze was cautiously cleaned, (using 10% ethanol solution).

Biochemical Estimation[22-24]

Preparation of Brain Tissue Samples

Mice were sacrificed on the final day of experiment. Dissected out the entire brain and separated subcortical region (with the stratum). Tissue was weighed and homogenized in 5 mL HCL–butanol solution for about one minute. Then sample was centrifuged for time 10 minutes. at 2000 rpm. After removing an (1-mL) aliquot from supernatant phase, it was taken in centrifuge tube holding 2.5 mL heptane and 0.31 mL 0.1M HCL. After 10 minutes the content in centrifuge tube was centrifuged for time 10 minutes at 2000 rpm to separate the two phases, and overlaying organic phase was removed. The aqueous phase (0.2 mL) was used for estimation of serotonin and gamma-aminobutyric acid (GABA).

Estimation of Serotonin

Standard Serotonin Preparation

Solutions of std. serotonin were prepared (in distilled water and HCl butanol 1:2) ranging from 0.01-1 µg/mL. Then read absorbance against blank (at 360–470 nm) in photofluorometer.

Estimation of Serotonin

To 0.2 mL of aq. extract (0.25 mL) O-Pthaldehyde was added. The fluorophore was developed by heating (to 100°C) for 10 minutes. The readings (for serotonin 360–470 nm) in the photofluorometer were taken after the samples had reached equilibrium (with the ambient temperature).

Estimation of GABA

GABA Assay

The aqueous phase of homogenate directly applied on the whatman filter paper. It was put as a solvent scheme on a chamber comprising n-butanol: acetic acid: water (12: 3: 5 v/v). It was removed and dried when the solvent reached the peak of the document. Similarly, at second time carried out after which the papers were sprayed by ninhydrin reagent and kept for 4–5 minutes in an oven at 100°C for drying. Then the portions which carry GABA corresponding with the standard were cut and soaked using solvent mixture of 0.005% $CuSo_4$ in 75% ethanol. Their absorbance was interpreted against blank at 550 nm for GABA in JASCO UV-visible-double beam spectrophotometer.

Calibration Curve

2.5 to 60 µg/mL solutions of standard GABA be prepared in 0.1N 80% ethanol. Then, these dilutions were used for paper chromatography. The spot cut portion obtained by running whatman spotted filter paper in n-butanol: acetic acid: water (12:3:5 v/v) as a solvent system and finally measured at 550 nm a in JASCO UV-visible-double beam spectrophotometer.

Statistical Analysis

Data were stated as mean ± SEM. The comparisons between the averages of series of values were done by one–way ANOVA test followed by Dunnett test.

Fig. 1: Preparation of EFEHU.

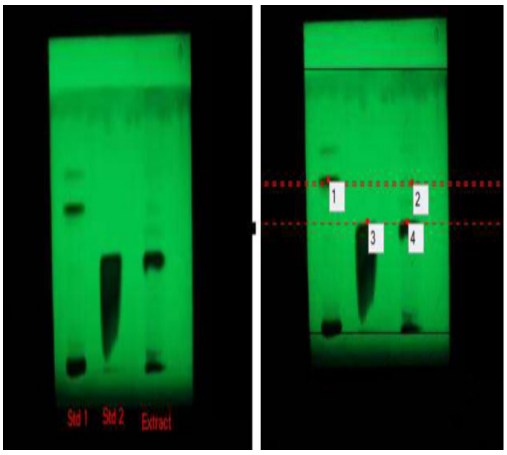

Fig. 2: HPTLC image.

Results

Average %yield of ethanolic fruit extract of *H. undatus* (EFEHU) was found to be 12.4% w/w. Phytochemical screening of EFEHU was carried out alkaloids, tannins, carbohydrates, flavonoids, terpenoids, saponins, oils, and phenolic compounds.

Qualitative Estimation of Quercetin and Gallic Acid by HPTLC Technique

The mobile phase used for HPTLC was toluene: ethyl acetate: GAA: formic acid: methanol [5: 3:1:1:1 drop].
In present research work qualitative estimation by HPTLC technique showed that presence of quercetin and gallic acid in EFEHU (Fig. 2, Table 1).
The EFEHU revealed significant antidepressant activity in both TST and DST model and anxiolytic activity in EPM test.

Evaluation of Antidepressant Activity

Evaluation of TST

In this test animal treated with two doses of EFEHU 200 mg/kg and 400 mg/kg showed significant ****p <0.0001 decease in immobility time on 7th day (148 ± 1.461, 143 ± 1.592), on 14th day (131 ± 1.826, 124 ± 1.125) and on 21st day (117.5 ± 1.565, 108.5 ± 1.232) respectively, when compared with control group on 7th day (170.5 ± 1.928), on 14th day (166.7 ± 1.667) and on 21st day (163.3 ± 1.145), respectively. Similarly, animal treated with imipramine (20 mg/kg) showed significant decrease in immobility time (117.3 ± 1.926, 111.2 ± 1.662, 102.2 ± 1.641) on 7th,14th and 21st day, respectively (Table 2).

Table 1: Qualitative estimation by HPTLC

Spot Description	*Rf value*
1. Standard quercetin	0.56
2. EFEHU	0.56
3. Standard gallic acid	0.41
4. EFEHU	0.41

Evaluation of Despair Swim Test

In this test animal treated with two doses of EFEHU 200 mg/kg and 400 mg/kg showed significant ****p <0.0001 decrease in immobility time on 7th day (124.2 ± 1.887, 120.3 ± 0.4216), on 14th day (118.7 ± 1.978, 115.8 ± 0.4773) and on 21st day (114.8 ± 1.973, 108.8 ± 1.138) individually after compared with control group on 7th day (139.3 ± 1.687), on 14th day (136.2 ± 1.447) and on 21st day (134.3 ± 1.563), respectively. Similarly, animal treated by imipramine (20 mg/kg) showed significant reduction in immobility time (112.3 ± 1.476, 108.7 ± 1.892, 98.17 ± 2.994) on 7th,14th and 21st day, respectively (Table 3).

Estimation of Srotonin in DST Model

Level of serotonin in tissue homogenate of brain in DST model were estimated. In this animal treated by 2 doses of EFEHU 200 mg/kg and 400 mg/kg presented significant rise in serotonin level ****p < 0.0001, respectively, when compared with control group (0.3912 ± 0.0169) likewise animal treated by imipramine (20 mg/kg) exhibited significant rise in serotonin level (Table 4).

Level of GABA in Tissue Homogenate of Brain in DST Model

The levels of neurotransmitter from tissue homogenate of brain were also measured. In this valuation animal treated by 2 doses of EFEHU 200 mg/kg and 400 mg/kg exhibited significant rise in GABA level (0.3269 ± 0.02779, 0.3547 ± 0.02779) **p < 0.001 when compared with control group (0.1602 ± 0.02779) likewise animal treated by imipramine (20 mg/kg) exhibited significant rise in GABA level (Table 5).

Evaluation of Anxiolytic Activity

Elevated Plus Maze Test

Effect of EFEHU on time spent in open arm in EPM apparatus

In this test animal treated by two doses of EFEHU 200 mg/kg and 400 mg/kg showed significant ****p < 0.0001 rise in time spent in the open arm (123.8 ± 0.7923, 144.5 ± 1.432) on 7th day and on 14th day (142.3 ± 1.054, 155.3

Table 2: Effect of EFEHU on Immobility period in TST

Group	*Treatment*	*Dose, Route of administration*	*TST Immobility time (s) (mean ± SEM)*		
			7th day	14th day	21st day
Control	Distilled water	10 mL/kg p.o.	170.5 ± 1.928	166.7 ± 1.667	163.3 ± 1.145
Standard	Imipramine	20 mg/kg p.o.	117.3 ± 1.926 **** (31.20%)	111.2 ± 1.662 ****(33.29%)	102.2 ± 1.641 **** (37.41%)
Test 1	EFEHU	200 mg/kg p.o.	148 ± 1.461 **** (13.19%)	131 ± 1.826 **** (21.41%)	117.5 ± 1.565 **** (28.04%)
Test 2	EFEHU	400 mg/kg p.o.	143 ± 1.592 **** (16.12%)	124 ± 1.125 **** (25.61%)	108.5 ± 1.232 **** (33.55%)

Table 3: Effect of EFEHU on Immobility period in DST

Group	*Treatment*	*Dose, Route of administration*	*DST -Immobility time (s) (mean ± SEM)*		
			7th day	14th day	21st day
Control	Distilled water	10 mL/kg p.o.	139.3 ± 1.687	136.2 ± 1.447	134.3 ± 1.563
Standard	Imipramine	20 mg/kg p.o.	112.3 ± 1.476 ****(19.38%)	108.7 ± 1.892 ****(20.19%)	98.17 ± 2.994 **** (26.90%)
Test 1	EFEHU	200 mg/kg p.o.	124.2 ± 1.887 **** (10.83%)	118.7 ± 1.978 **** (12.84%)	114.8 ± 1.973 **** (14.51%)
Test 2	EFEHU	400 mg/kg p.o.	120.3 ± 0.4216 **** (13.63%)	115.8 ± 0.4773 **** (14.97%)	108.8± 1.138 **** (18.98%)

Table 4: Level of serotonin in tissue homogenate of brain in DST model

Group	*Treatment*	*Dose, Route of administration*	*Serotonin (µg/mL) (mean ± SEM)*
Control	Distilled water	10 mL/kg p.o.	0.3912 ± 0.0169
Standard	Imipramine	20 mg/kg p.o.	0.8558 ± 0.01558 ****
Test 1	EFEHU	200 mg/kg p.o.	0.67 ± 0.01558 ****
Test 2	EFEHU	400 mg/kg p.o.	0.7882 ± 0.01133 ****

Table 5: Level of GABA in tissue homogenate of brain in DST model

Group	*Treatment*	*Dose, Route of administration*	*GABA (µg/mL) (mean ± SEM)*
Control	Distilled water	10 mL/kg p.o.	0.1602 ± 0.02779
Standard	Imipramine	20 mg/kg p.o.	0.4659 ± 0.04813 ***
Test 1	EFEHU	200 mg/kg p.o.	0.3269 ± 0.02779 **
Test 2	EFEHU	400 mg/kg p.o.	0.3547 ± 0.02779 **

± 0.106), respectively, after compared with control group (35.83 ± 0.5426) on 7th day and on 14th day (49 ± 1.751). Similarly, animal treated with diazepam (2 mg/kg) exhibited significant rise in time spent in the open arm (153.5 ± 1.176, 172.8 ± 1.249) on 7th and 14th day, respectively (Table 6).

Effect of EFEHU on no. of Entries into Open Arm in EPM Apparatus

In this test animal treated by two doses of EFEHU 200 mg/kg and 400 mg/kg showed significant ****$p < 0.0001$ rise in no. of entries in open arm (9.667 ± 0.7601, 10.83 ± 0.7032) on 7th day and on 14th day (11.67 ± 0.7601, 14 ± 0.5774), respectively, compared with control group (3.667 ± 0.4944) on 7th day and on 14th day (4.333 ± 0.3333) Similarly, animal treated with diazepam (2 mg/kg) showed significant rise in number of entries in open arm (12.5 ± 0.7638, 17.67 ± 0.8433)on 7th & 14th day, respectively (Table 7).

Level of Serotonin in Tissue Homogenate of Brain in EPM Test

The levels of neurotransmitter from tissue homogenate of brain were determined. In this valuation animal treated by 2 doses of EFEHU 200 mg/kg and 400 mg/kg showed significant rise in serotonin level ****$p < 0.0001$ respectively, when compared with control group (0.2798 ± 0.01577) likewise animal treated by diazepam (2 mg/kg) exhibited significant increase in serotonin level (Table 8).

Level of GABA in Tissue Homogenate of Brain in EPM Test

The neurotransmitter level from tissue homogenate of brain were determined. In this valuation animal treated by 2 doses of EFEHU 200 mg/kg and 400 mg/kg showed significant rise in GABA level ***$p < 0.0005$, ****$p < 0.0001$, respectively, when compared with control group (0.165 ± 0.02556) likewise animal treated by diazepam (2 mg/kg) exhibited significant rise in GABA level.

Values were stated in a mean ± SEM. The results were statistically analyzed by the one-way ANOVA (followed by Dunnett's test) (n = 6). ****$p < 0.0001$ as compared with control group. The values in bracket indicate that the % reduction in immobility time (Table 9).

Values were expressed in a mean ± SEM. The results were statistically analyzed by the one - way ANOVA (followed by Dunnett's test) (n = 6). ****$p < 0.0001$ as compared to control group. The values in bracket indicates that the % reduction in immobility time.

Table 6: Effect of EFEHU on time spent in open arm in EPM apparatus

Group	*Treatment*	*Dose, Route of administration*	*Time spent into open arms (s) (mean ± SEM)*	
			7^{th} day	14^{th} day
Control	Distilled water	10 mL/kg p.o.	35.83± 0.5426	49 ± 1.751
Standard	Diazepam	2 mg/kg i.p.	153.5 ± 1.176 ****	172.8 ± 1.249 ****
Test 1	EFEHU	200 mg/kg p.o.	123.8 ± 0.7923 ****	142.3 ± 1.054 ****
Test 2	EFEHU	400 mg/kg p.o.	144.5 ± 1.432 ****	155.3 ± 0.106 ****

Table 7: Effect of EFEHU on no. of entries into open arm in EPM apparatus

Group	*Treatment*	*Dose, Route of administration*	*No. of entries into open arms (mean ±SEM)*	
			7^{th} day	14^{th} day
Control	Distilled water	10 mL/kg p.o.	3.667 ± 0.4944	4.333 ± 0.3333
Standard	Diazepam	2 mg/kg i. p.	12.5 ± 0.7638 ****	17.67 ± 0.8433 ****
Test 1	EFEHU	200 mg/kg p.o.	9.667 ± 0.7601 ****	11.67 ± 0.7601 ****
Test 2	EFEHU	400 mg/kg p.o.	10.83 ± 0.7032 ****	14 ± 0.5774 ****

Table 8: Level of serotonin in tissue homogenate of brain in EPM test

Group	*Treatment*	*Dose, Route of administration*	*Serotonin (µg/mL) (mean ± SEM)*
Control	Distilled water	10 mL/kg p.o.	0.2798± 0.01577
Standard	Diazepam	2mg/kg i.p.	0.7159 ± 0.02163 ****
Test 1	EFEHU	200 mg/kg p.o.	0.5021 ± 0.01577 ****
Test 2	EFEHU	400 mg/kg p.o.	0.5962 ± 0.01873 ****

Table 9: Level of GABA in tissue homogenate of brain in EPM test

Group	*Treatment*	*Dose, Route of administration*	*GABA (µg/mL) (mean ± SEM)*
Control	Distilled water	10 mL/kg p.o.	0.165 ± 0.02556
Standard	Diazepam	2mg/kg i.p.	0.4561 ± 0.01754 ****
Test 1	EFEHU	200 mg/kg p.o.	0.2898 ± 0.01754 ***
Test 2	EFEHU	400 mg/kg p.o.	0.3314 ± 0.01386 ****

Values are stated in a mean ± SEM. Statistical analysis of data was completed by one -way ANOVA (followed by Dunnett's test) (n = 6). ****p < 0.0001 as compared to control group.

Values are stated in a mean ± SEM. Statistical analysis of data was carried out by one -way ANOVA (followed by Dunnett's test) (n = 6). ***p< 0.0001, ** p< 0.001 compared with control group.

Values are stated in a mean ± SEM. The statistical analysis of data was completed by one -way ANOVA (followed by Dunnett's test) ****p < 0.0001 as compared to control group.

Values are stated in a mean ± SEM. Statistical analysis of data was completed by one -way ANOVA (followed by Dunnett's test.) ****p < 0.0001 as compared to control group.

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Values are stated in a mean ± SEM. Statistical analysis of data was completed by one -way ANOVA (followed by Dunnett's test) (n = 6). ****p < 0.0001, ***p < 0.0005 as compared to control group.

Discussion

Depression patients often have sign of anxiety disorder. Together both depression and anxiety disorders may occur and meet both criteria. Discrimination between them can be difficult but it is vital to detect and to treat both diseases. In order to facilitate better mental health outcomes, experts are well placed to identify and play a primary role in treating of these diseases.[25]

Chronic use (particularly chemical synthesized) of available pharmacological agents is linked with serious adverse events. Therefore, there is a necessity of safe, alternative, as well as efficient way of dealing with different disorders. Now to the usage of the herbal compounds, nutrients, and supplements as therapy choice than conventional food for convenient therapy.[13]

The primary phytochemical study revealed in EFEHU presence of alkaloids, tannins, glycoside, phenolic compound, flavonoids, saponins and steroids. These are important for antidepressant and anxiolytic activity. It has been reported that quercetin[26] and gallic acid[27] showed antidepressant and anxiolytic activity and in present research work qualitative estimation by HPTLC technique showed that presence of quercetin and gallic acid in EFEHU.

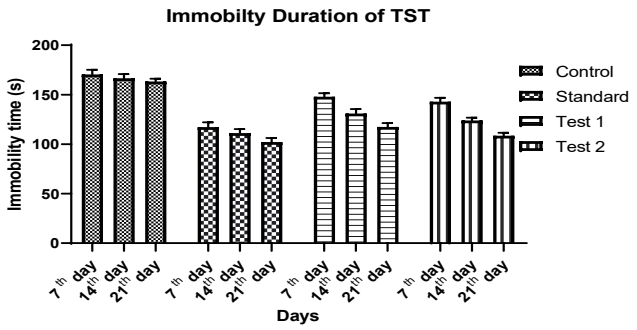

Fig. 3: Effect of EFEHU on immobility period in TST

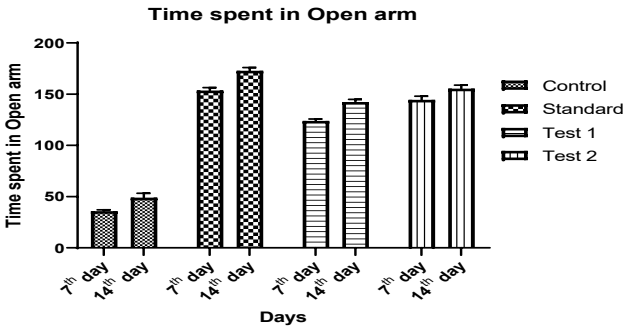

Fig. 7: Effect of EFEHU on time spent in open arm in EPM apparatus

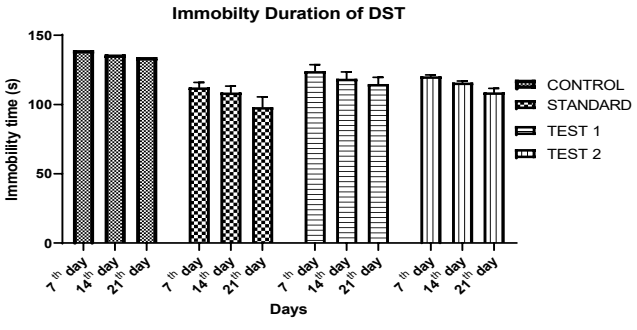

Fig. 4: Effect of EFEHU on immobility period in DST

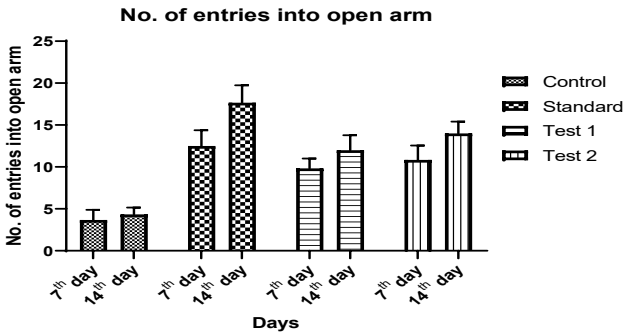

Fig. 8: Effect of EFEHU on no. of entries into open arm in EPM apparatus

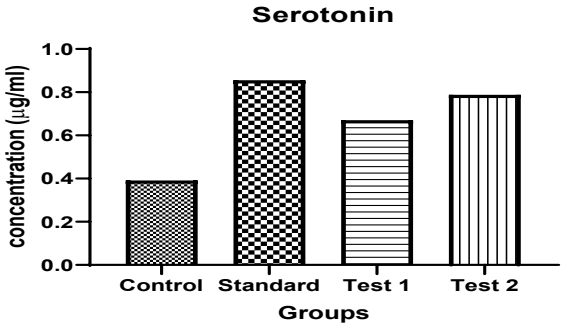

Fig. 5: Effect EFEHU on Level of Serotonin in tissue homogenate of brain in DST model

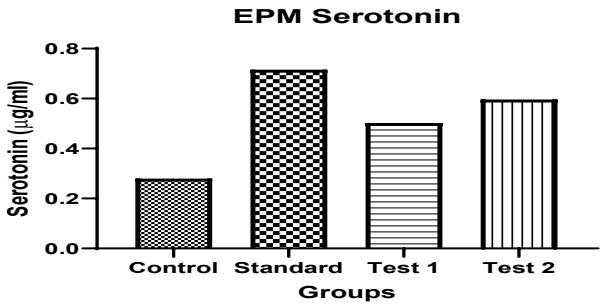

Fig. 9: Effect of EFEHU on Level of Serotonin in tissue homogenate of brain in EPM test

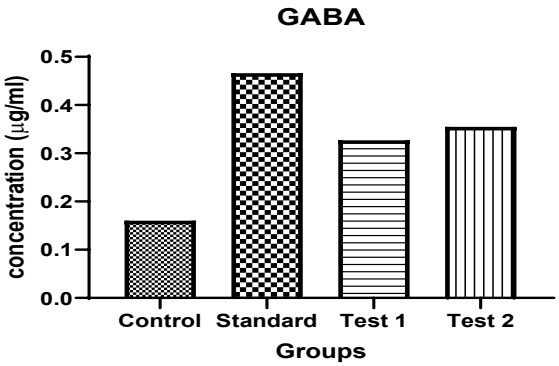

Fig. 6: Effect EFEHU on Level of GABA in tissue homogenate of brain in DST model

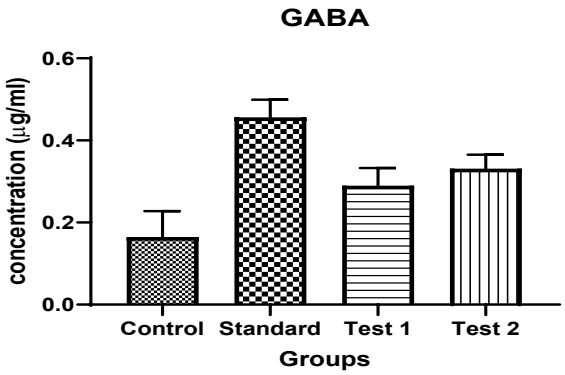

Fig. 10: Effect of EFEHU on Level of GABA in tissue homogenate of brain in EPM test

Antidepressant Activity

In TST and DST model on days of 7th, 14th and 21st we observed that the EFEHU significantly (****$p < 0.0001$) reduced immobility time in both DST and TST in comparison with control and revealed a dose dependent antidepressant activity as shown in previous work by Talha Jawaid *et al.*[28] In TST model the percentage decrease in immobility of test 1 (13.19%, 21.41%, 28.04%), Test 2 (16.12%, 25.61%, 33.55%) and standard group (31.20%, 33.29%, 37.41%) compared with control group on 7th, 14th and 21st day, respectively (Fig. 3). In this test, the characteristic behavior assessed, termed immobility, was considered to reflect despair behaviour as seen in the depressed human, and therefore any decrease in this parameter reflects the activity of an antidepressant. The antidepressant medicine's clinical efficacy is significantly correlated with their potency in DST. In DST model, the percentage decrease in immobility of test 1 (10.83%, 12.84%, 14.51%), Test 2 (13.63%, 14.97%, 18.98%) and standard (19.38%,20.19%,26.90%) as compared with control group on 7th, 14th, 21st day, respectively (Fig. 4). Remarkably, our data specify that higher doses of EFEHU in both DST and TST have been more effective than smaller doses.

The most prevalent theory for depression pathogenesis is "monoamine hypothesis". The central mono amines deficiency like noradrenaline, 5-hydroxy tryptamine, and dopamine are responsible for the signs of depression. Many currently used antidepressants act by elevating the concentration of the neurotransmitters in the brain.

In this study the biogenic amine level estimated in despair swim test and result exhibited that the serotonin level was increased significantly (****$p < 0.0001$) and dose-dependently in 200 mg/kg and 400 mg/kg EFEHU treated group, respectively as compared with control group (Fig. 5). Standard drug imipramine also showed increase in serotonin level. As shown in previous study by Wang *et al.*[29]

The CNS main inhibitory neurotransmitter is GABA and GABAergic dysfunction causes mood disorders or neurological ailments such as seizures, anxiety, and depression. Recent evidence has suggested GABAergic system linking with depression. Similarly, in the somatic antidepressant therapies mechanism of action also. In the animal frontal cortex particularly, $GABA_B$ receptors were found to be increased following chronic antidepressant treatments. Most strikingly, a reduced GABA level in plasma and cerebrospinal fluid and in occipital cortical brain was found in patients with major depression. Thus, a GABAergic system deficit appears to contribute both anxiety and depression. In EFEHU seems to be part of the therapeutic effects in both anxiety and depression in mice.[29]

In present study for DST model, level of GABA was significantly increased (** $p < 0.001$) in dose dependent manner as compared to the control group but less than that of standard drug Imipramine (***$p < 0.0001$) as shown in previous work by Talha Jawaid *et al*. (Fig. 6).[48]

EFEHU contain chemical constituent are kaempferol, quercetin, b-sitosterol, isorhamnetin, gallic acid, betacyanin, alkaloids, terpenoids, glycoside, flavonoids, tannins, steroids and phenolic compounds, saponins, proteins, carbohydrate, amino acids, vitamins B1, B2, B3, B6, C and minerals like copper, calcium, iron, phosphate, magnesium and might be responsible for antidepressant activity.

Anxiolytic Activity

EPM is presently the first choice for an anxiolytic drug test. In this work, we observed that EFEHU (200 and 400 mg/kg) induced a significant rise in the no. of entries and total time spent in open arms, on 7th and 14th days. (****$p < 0.0001$) (Fig. 7, 8). The activity was slightly lower than the standard diazepam used as similarly shown in previous studies reported by Nanna *et al.* [4]

Anxiety may be caused because of the involvement of neurotransmitters and transporters of serotonergic, noradrenergic, glutaminergic, and GABAergic neurons and hormones (neuropeptide Y, CCK). Decreased GABA level is vital in CNS in producing anxiety. GABA release and 5 HT also play a major part in anxiety development.[30]

In the present EPM test the serotonin level is significantly increased (****$p < 0.0001$) in EFEHU treated group dose-dependently (200 mg/kg and 400 mg/kg, respectively) as compared to the control group (Fig. 9). The GABA level in present study significantly increased (****$p < 0.0001$) in EFEHU treated group dose-dependently (200 mg/kg and 400 mg/kg, respectively) as compared to the control group (Fig. 10) as shown in previous work by Talha Jawaid *et al.*[28]

The mechanism of action through which EFEHU exerts both activities was not clear by performing this experimental test. However, an increase in serotonin (5-HT) and GABA level could be a possible reason for the reported antidepressant and anxiolytic effects. The effects reported here may result from one chemical substance or different plant secondary metabolites.

Conclusion

The study found that, for antidepressant activity, the group treated with ethanolic fruit extract of *H. undatus* (EFEHU) shows decreased immobility time in TST and FST. For anxiolytic activity in EPM increased time spent in open arm and no. of entries into open arm.

The estimation of rodent's brain-free amino acids revealed that the antidepressant and anxiolytic potential of EFEHU may be related with an increase in brain serotonin and GABA concentration in both DST (antidepressant activity) and EPM (anxiolytic activity) models. The feasible mechanism could be due to its phytochemical compounds

or modulatory effect on central neurotransmitter system. In addition, studies are required to examine its mechanism of action and the active principle responsible for antidepressant and anxiolytic action. These results can be advanced to develop novel antidepressant and anxiolytic drugs with better effectiveness and less side effects.

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