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Analysing the antidepressant and drug efflux competence of *Clitoria ternatea* L. as P-glycoprotein inhibitor to facilitate blood brain barrier

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ABSTRACT. *Clitoria ternatea* L. is a vital ayurvedic herb featured with a wide spectrum of mental health benefits. The present study investigates the competence of the plant against depression and to inhibit the membrane efflux protein P-glycoprotein (P-gp) that can regulate and restrict drug permeation into the brain. Antidepressant competence of the aqueous plant extract was assessed by animal despair studies on depression induced female mice models. The P-glycoprotein inhibitory potential of active phytocompounds was evaluated by molecular docking assay and substantiated by a cell line study. The *in vivo* studies exhibited a significant difference in the immobility time thereby establishing antidepressant activity. The histopathological sections from cortex region of treated brain showed decreased degenerative changes. Ten imperative phytocompounds facilitated docking complexes against P-glycoprotein among which Kaempferol 3-O-(2″,6″-di-O-rhamnopyranosyl) glucopyranoside exhibited a finest docking score of -12.569 kcal mol^{-1}. Conversely it was attested by the rhodamine transport assay that demonstrated the inhibitory potential to surpass blood brain barrier. The outcome of the study endorses the efficacy of *Clitoria ternatea* L. as an idyllic brain drug and facilitates brain permeability.

Keywords: Mental health; Ayurvedic herb; Kaempferol 3-O-(2″,6″-di-O-rhamnopyranosyl) glucopyranoside; Animal despair studies; Rhodamine transport assay; Molecular docking.

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Introduction

Emotional disturbance is an indistinct condition of mind which becomes unbalanced and leads to several neurological disorders where, depression is one perilous mental ailment. Incidence of depressive mood disorders is rising in the modern stressful society leading to increased risks of self- harm or suicide which is a significant contributor to the global burden of disease (World Health Organization [WHO], 2015). Traditional system of medicine has a deep rooted contextual derived from ancient communities that endorses antiquity than western medicine, but it requires a profuse attempts to combine the best of different healing traditions to meet the challenge faced in healthcare requirements of modern era (Perinchery, 2013). Shankhpushpi is a reputed drug constituted in medhya rasayana and according to the pharmacopoeia of India, *Convolvulus pluricaulis* (Convulvulaceae) as a whole plant rightfully claim the name of Shankapushpi. Ayurvedic practitioners have used three other medicinal herbs such as *Canscora decussate* Schult. (Gentianaceae), *Evolvulus alsinoids* Linn. (Convulvulaceae) and *Clitoria ternatea* Linn. (Papilionaceae) as remedy for mental ailment (Nahata, Patil, & Dixit, 2010; Nahata, Patil, & Dixit, 2008). Together, all these four medicinal herbs are also known as shankpushpi.

Clitoria ternatea L. commonly known as Butterfly pea belonging to the Leguminosae (Fabaceae) being an imperative constituent requires distinct research to scrutinize its therapeutic value (Taranalli & Cheeramkuzhy, 2000).The vital phytocompounds has the potential to inhibit depression inducing proteins (MAO A & B) Monoamine Oxidase A and B (Margret, Begum, Parthasarathy, & Suvaithenamudhan, 2015) and can be employed as a lead for developing new phytoceuticals for the treating human ailment to promote neurological health by increasing memory and activity of neurotransmitters (Rai et al., 2002; Mukherjee, Kumar, & Houghton, 2007).

The presence of physiological barriers effort to hinder the therapeutic molecules to the targeted sites and drugs targeting central nervous system (CNS) are being challenged by the complicated mechanism of the blood brain barrier (BBB) which is considered as a hurdle that results in the inability of therapeutic compounds to the targeted site. P-glycoprotein (P-gp) is an ATP-binding cassette (ABC) membrane transporter acting as a drug efflux pump which is expressed in many tissue barriers and secretary epithelia (Sugimoto et al., 2011). Most often it acts as an efflux transporter limiting cellular and tissue entry of endogenous toxins and xenobiotics, or facilitating their removal from target organs (Wohlfart, Gelperina, & Kreuter, 2012). P-gp activity can be inhibited by a wide range of drugs that are termed as inhibitors and can lead to drug–drug interactions (Fenner et al., 2009). This is due to the impairment of pharmacokinetics of a drug substrate that causes the P-gp inhibitory potential which remains as demand in several pharmaceutical industries that has the potential to cure several ailments (International Transporter Consortium et al., 2010).

The present study aims to explore the antidepressant activity of *Clitoria ternatea* L. and augment its potential as a competent brain drug against the targeted membrane efflux protein P-gp that counteracts depression and stabilizes mental health.

Material and methods

Chemicals

Kaempferol-3-monoglucoside, Malvidin-3- O –glucoside, Dulbecco's modified Eagle medium (DMEM) and Rhodamine 123 was purchased from Sigma-Aldrich (Merck) Bengaluru, India. The standard antidepressant drug (Vemlafaxin) and P-gp inhibitor (Verapamil) was obtained from Cadila Pharmaceuticals, (Ahmedabad, India). All other chemicals and solvents were of analytical reagent grade.

Plant material and aqueous extract preparation

The leaves of *Clitoria ternatea* L., family Fabaceae (Figure 1) were collected from river banks of Uyyakondan Thirumalai, Tiruchirappalli, Tamilnadu, India. The leaf extract was obtained by adapting a modified method described by Umeh, Oluma, and Igoli (2005). The samples were cleaned, shade dried and powdered. 100 grams of powdered leaf sample were weighed and boiled with 1 L of distilled water for 10 min. at 70ºC. The plant extract was filtered and evaporated to dryness and further used for analysis. The plant's identity was confirmed anatomically and morphologically based on its pharmacopoeial monograph at the Rapinat Herbarium and Centre for Molecular Systematic, St. Josephs College (Autonomous), Tiruchirappalli, Tamilnadu, India where, the voucher specimen number (AAM 001) was assigned and deposited.

Class	**Magnoliopsida**
Sub Class	**Rosidae**
Order	**Fabales**
Family	**Fabaceae**
Genus	**Clitoria**
Species	**ternatea.**
Botanical name	***Clitoria ternatea* L.**
Common name	**Asian pigeon wings**

Figure 1. Pictorial representation of the twinner *Clitoria ternatea* L with its taxonomical classification

In vivo animal despair studies

Animals

The experiment was carried out on 30 naïve adult female albino Swiss mice weighing 22–25 g. The animals were housed in the environmentally controlled rooms (temperature maintained at 24± 3°C and humidity 40–60%) in standard cages in groups of 4 as positive control (depressive without treatment), negative Control (normal) and experimental group 1 (Standard drug) and experimental group 2 (Plant extract) with unlimited access to water and food. The rooms were illuminated with a 12 hours light/dark cycle. The procedures began after acclimation period in the laboratory conditions. All procedures were conducted in accordance with the institutional ethical Committee for the Purpose of Control and Suspension of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi. The procedures and protocols were approved by the Ethics Committee at the department of animal sciences, Bharathidasan University, Tiruchirappalli (Ref No.:BDU/IAEC/2014/NE/39).

Induction of depression and drug administration

Excluding the test group of animals, replicated animal despair pattern was carried out in all the models regularly for two weeks through which depression was induced. The procedure persisted during the test sessions on 1st, 3rd, 5th and 7th day and the behavioral patterns were recorded to assay the immobility period. Pursuing this the treatment phase is scheduled for a week and eventually (14th day) the animal is sacrificed for histopathological assessment. Venlafaxine (20 mg kg^{-1} p.o.) was dissolved in 0.9% NaCl and plant aqueous extract (50 mg kg^{-1} p.o.; 100 mg kg^{-1} p.o) were considered as test solutions, whereas, saline as control solution were administered orally (p.o = per orem) 30 min. prior behavioral testing. The volume of all administered solutions/suspension was 0.01 mL g^{-1}. The doses and treatment schedules were selected on the basis of literature and the results of our previous experiments (Margret, Dhayabaran, & Kumar, 2017; Dhayabaran & Margret, 2017).

Forced Swim Test (FST)

FST was carried out according to the method of Porsolt, Anton, Blavet, & Jalfre (1978). Each animal was placed individually for 6 min. into a glass cylinder (height 25 cm, diameter 10 cm) with 15 cm of water at 23–25 °C. After the first 2 min. of the test, total duration of immobility (in seconds) was measured. The mice are considered to be immobile when it stopped struggling and remained floating motionless. The water was habitually changed after each test.

Tail suspension test (TST)

TST was carried out according to the method of Cryan, Monbereau, and Vassout (2005). Each mouse was suspended for 6 min. by the tail (2 cm from the end of the tail) using adhesive tape. After the first 2 min. of the test, total duration of immobility (in seconds) was measured. Immobility was determined when the animal ceased moving limbs and body.

Histological procedure

Subsequent to behavioral studies with an interval of 2 hours both the normal and treated groups were sacrificed for dissection. The secluded, brain regions were post-fixed with formalin (10%) for 7days, dehydrated, cleared and embedded in paraffin according to routine histological procedures. The test samples were sliced in the frontal plane in 7 µM consecutive sections and the cortex region was subjected to histopathological studies.

Molecular docking study against the membrane efflux protein P-gp

In silico molecular docking of ten imperative phytocompounds of *Clitoria ternatea* L. against the targeted protein P-gp was carried out using Glide software (Schrodinger Inc. U S A- Maestro version 10.2) (Friesner et al., 2006). The crystallographic coordinates for target protein P-Glycoprotein was retrieved from the Protein Data Bank (PDB ID 4M1M). The data set of compounds were taken from literature reported by (Tiwari & Gupta, 1959; Ripperger, 1978). The 3D structures of the phytocompounds considered as ligands were retrieved and downloaded as .mol files from the site of PubChem database.

The small molecules were prepared using the LigPrep wizard from Schrödinger (Greenwood et al., 2010) by assigning the bond orders and bond angles and then subjected to minimization using OPLS_2005 force field (Greenwood, Calkins, Sullivan, & Shelley, 2010). For accurate enumeration of small molecules protonation states in biological condition we used Epik (Shelley et al., 2007). Grid generated output file was uploaded as an input for Ligand docking against protein prepared targets in GLIDE.XP mode was adopted by selecting Flexible docking mode (QikProp, 2015). The module is performed on minimized structures to calculate the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties. This accesses the disposition and potential toxicity of ligand with in an organism along with the overall pharmacological properties of these molecules that justify them as biologically active.

Cell Line and Culture Conditions

hCMEC/D3 (Poller et al., 2013) cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum,2 mM L-Glutamine, 44mM $NaHCO_3$, 22.7 mM glucose, 10ml l^{-1} non-essential amino acid solutions, 100U Penicillin –G and 100µg ml^{-1} streptomycin. The cells were cultivated in 5% CO_2 with a humidified atmosphere at 37ºC. Cells were grown on 12-well plates at a density of 37,000 cells cm^{-2}.

Functional assay for P-glycoprotein

The activity of P-glycoprotein was evaluated by measuring intracellular accumulation of rhodamine 123 in hCMEC/D3. The assay was based on the inhibitory potential of the phytocompounds that estimates the polarity and release of fluorescent dye rhodamine 123, which is a substrate of P-gp (Haseloff, Blasig, Bauer, & Bauer, 2005). Cells were incubated at 37ºC with 5.25 µM rhodamine 123 for 30 min., in the presence or absence of various concentrations of P-gp inhibitors (Kaempferol-3-monoglucoside, Malvidin-3- O –glucoside 10mg ml^{-1} and with standard P-gp Inhibitor Verapamil (50µM) as a positive control. After washing in phosphate-buffered saline, cells were lysed in distilled water, and intracellular levels of rhodamine 123 were by fluorescence microplate reader. (BioTek, USA; excitation wavelength at 485, emission wavelength at 538 nm). Data were expressed as % of rhodamine 123 accumulation in control cells not exposed to P-gp inhibitors, arbitrarily set at 100%.

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences [SPSS] (2008) version 17.0.

All the data represent mean ± S.E.M. values and the statistical difference of control and the test data were analyzed by means of one -way analysis of variance (ANOVA) followed by multiple range test (Tukey's test). Differences at $p < 0.05$ were considered to be statistically significant.

Results

Behavioural studies to access the antidepressant activity of *Clitoria ternatea* L.

Animal despair tests are the most commonly used preclinical paradigms for predicting anti-depressant activity of drug after their acute administration. The in vivo study aims to evaluate the therapeutic effectiveness of *Clitoria ternatea* L. against depression by employing behavioural studies like force swim and tail suspension test in mice. This is considered as a preliminary study to attest the synchronized potential of phytocompounds present in the crude (aqueous) leaf extract. Immobility in forced swim and tail suspension test showed significant impairment before treating with both the standard drug and plant extract on 7th day as compared to 1st day. However, immobility score after the treatment was less in contrast to the untreated group. The results of immobility time have been presented in Table 1 and 2. Where the mean values of immobility of all treatments was found to be significant specifically on the 7th day (Figure 2A & B) in comparison to the 1st day immobility time and with the disease control ($p \leqslant 0.05$). The animal modeling study endorses the effectiveness of the aqueous extract belonging to the plant *Clitoria ternatea* L.

Table 1. Effect of *Clitoria ternatea* L. on the duration of Immobility time in Force Swimming Test using mice models

Groups & Days	Depressed	Normal	Standard drug	Plant Extract (50 mg kg^{-1})	Plant Extract (100 mg kg^{-1})
Day 1	165.1±4.0	-	164.8±3.1	161.5±3.01	162.8±2.1
Day 3	178.8±6.9	-	175.1±2.7	174.3±2.1	171.6±1.7
Day 5	193.5±4.8	-	194.3±1.5	189.3±4.1	189.5±3.0
Day 7	201.3±8.3	-	202.3±3.7	203.0±6.3	203.1±5.2
Day 14	199.5±7.3*	52.5± 1.87*	58.0±2.8*	106.5±2.07*	84.8±5.7*

*Values represented mean S.E.M. (n=6), $p < 0.05$ Vs Disease Control

Table 2. Effect of *Clitoria ternatea* L. on the duration of Immobility time in Tail Suspension Test using mice models

Groups & Days	Depressed	Normal	Standard drug	Plant Extract (50 mg kg^{-1})	Plant Extract (100 mg kg^{-1})
Day 1	166.3±1.7	-	162.0 ±2.5	159.5±2.3.	162.8±1.8
Day 3	182.1±3.6	-	178.2±4.2	181.3±4.04	185.6±2.7
Day 5	191.0±5.2	-	187.00±4.1	197.3±4.4	199.3±4.9
Day 7	206.3±4.4	-	200.5±6.5	208.5±5.7	206.8±3.6
Day 14	218.5±4.0*	83.1± 0.93	88.2±6.4*	138.8±6.43*	102.8±3.3*

*Values represented mean S.E.M. (n=6), $p < 0.05$ Vs Disease Control

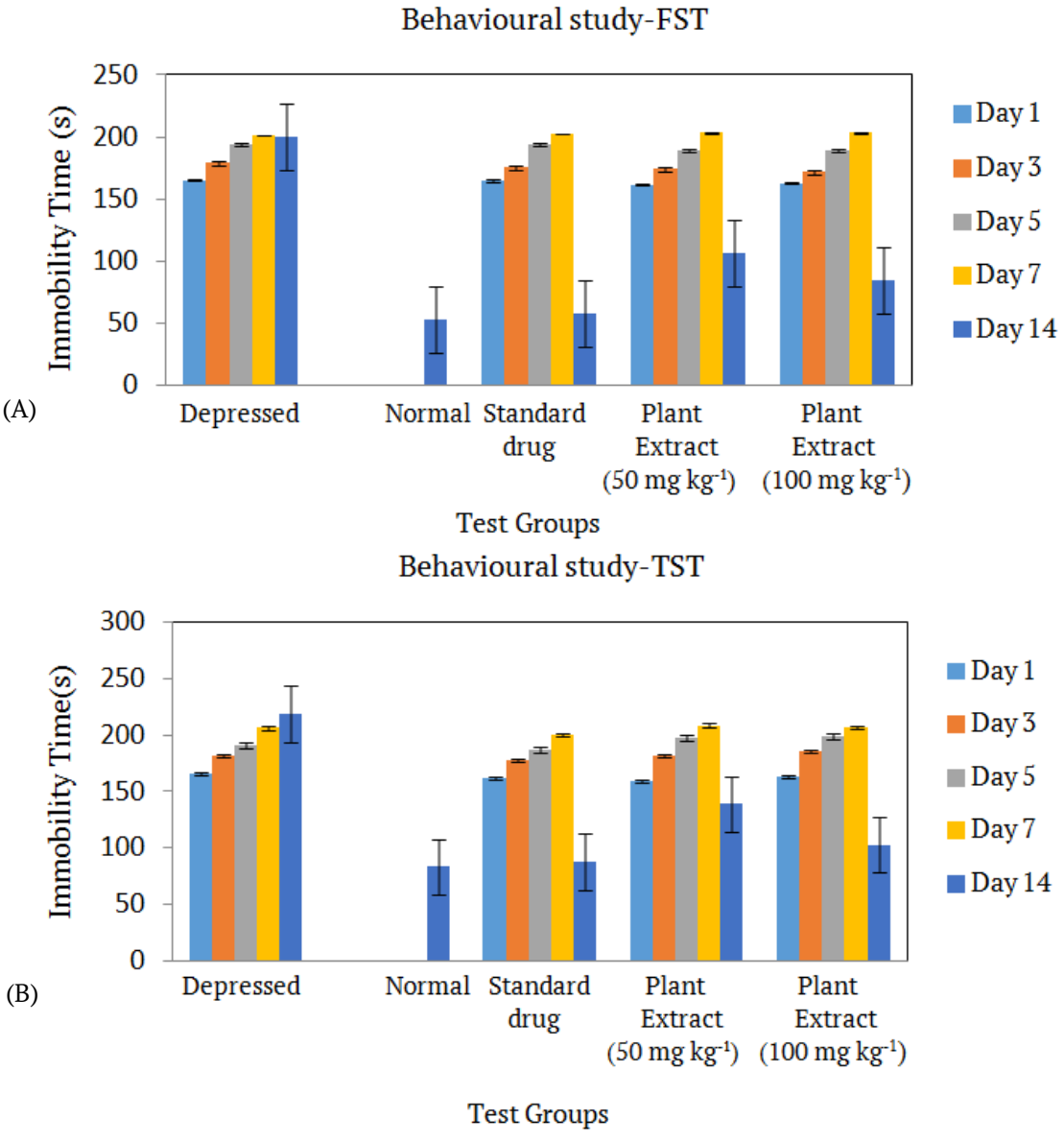


Figure 2. Effect of aqueous leaf extract (*Clitoria ternatea* L.) in assaying the immobility time through despair studies A. (FST) B. (TST) in depression induced mice.

Values are expressed as mean ± SEM *p ⩽ 0.05 as compared to disease control in specific with the 7th day of immobility time:

Histopathological examination on cerebral cortex

Histological studies were performed in the forebrain region (cerebral cortex) of the normal, depressed and treated animals. Figure 3A (i-iv) shows the histopathological conditions of the depressed mice such as (CE) Cellular Edema, (G) Gliosis, (LI) Lymphatic Infiltration and (FI) Fatty Infiltration. The depressed histology of the animals illustrates increase patterns of degenerative changes, gliosis, cellular edema, Fatty infiltration and infiltration in the lymphatic cells and perivascular areas. Subsequently Figure 3B (i and ii) depicts the normal mice which were not depressed and the treated mice with the plant extract. Less and mild changes are observed in the cerebral cortex regions of the treated mice with the plant extract, which exemplifies the significance of the plant *Clitoria ternatea* L. as an efficient brain drug that has the ability to reverse the degenerative changes, gliosis and cellular edema and can reinstates psychological illness.

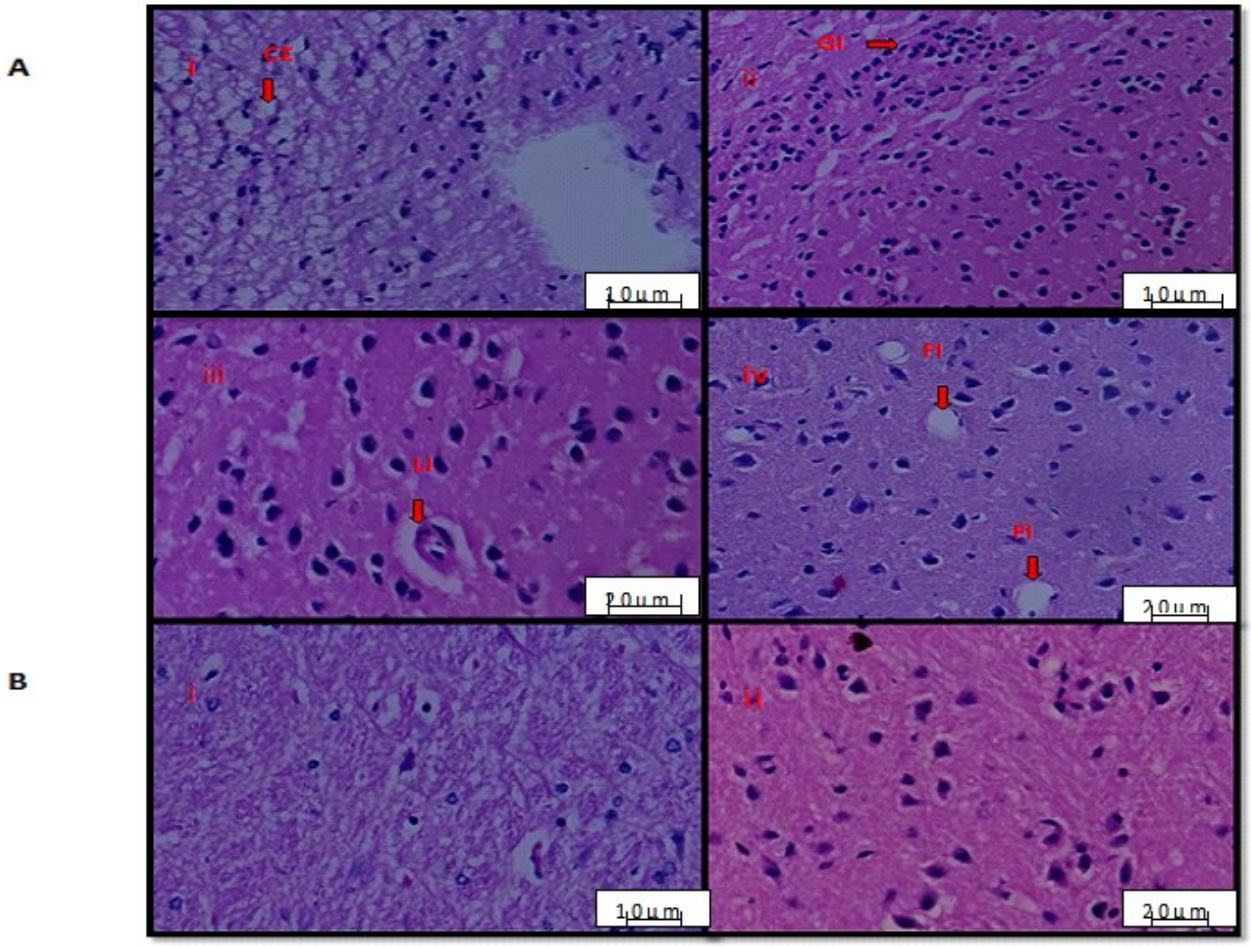
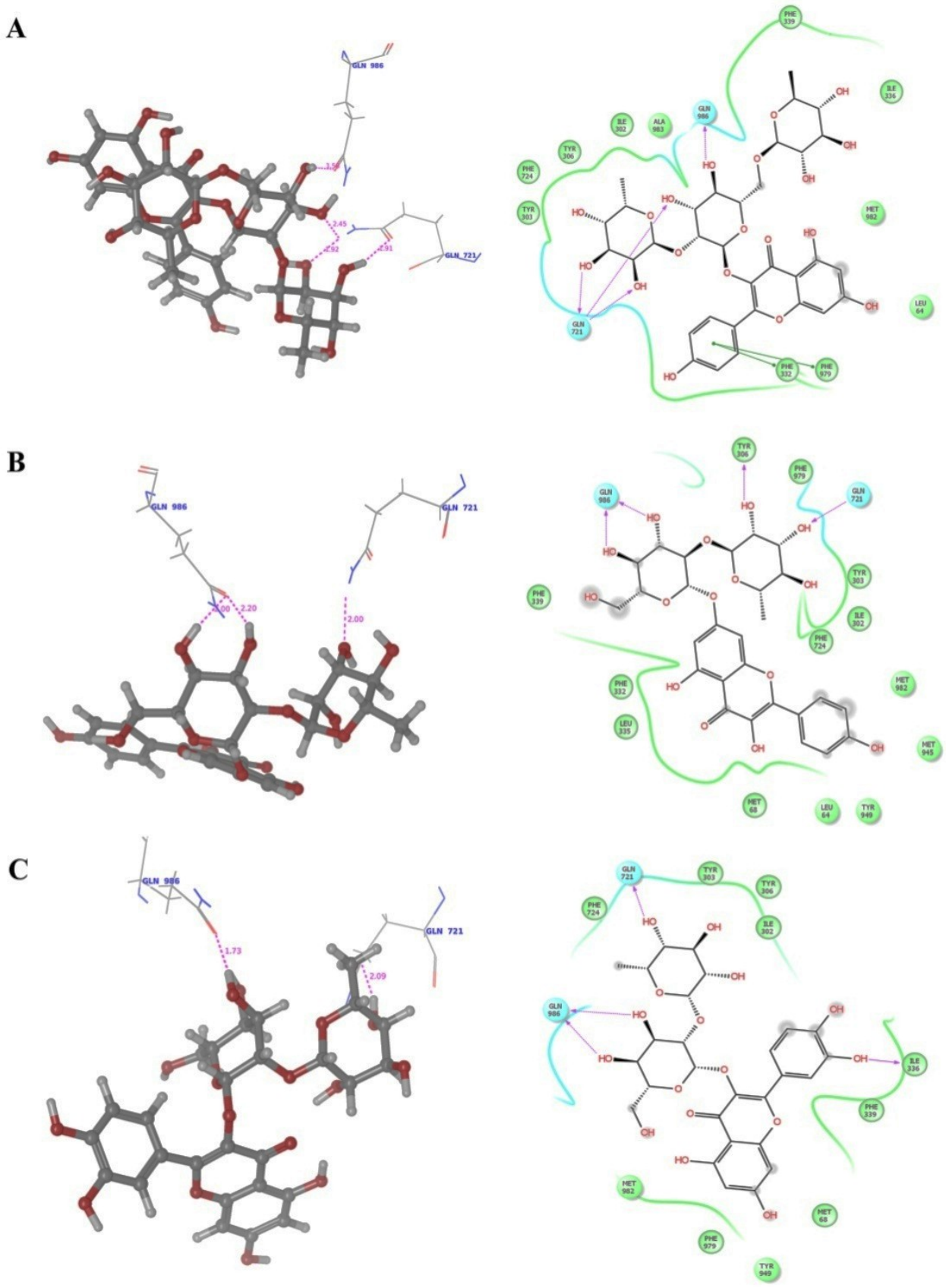


Figure 3. Histopathological changes and appearances of cerebral cortex regions of the A. depression induced mice (i) CE-Cellular Edema, DC-Degenerative Changes. (ii) G-Gliosis, IP-Infiltration in perivascular area (iii) LI-Lymphatic Infiltration (iv) FI -Fatty Infiltration B.(i)Normal and (ii) Treated mice with aqueous leaf extract (100mg kg^{-1} weight, p.o)

In silico analysis to assay the attributes of *Clitoria ternatea* L. as a permeable drug targeting the Blood Brain Barrier

Contemporary research emphasizes on active compounds that targets specific ailments. Though the antidepressant competence of *Clitoria ternatea* L. is renown there is a need to identify the phytocompounds that can fortify it as a brain drug to contest the blood brain barrier. Therefore docking studies were performed with ten bioactive molecules against the molecular target (P-gp) based on their corresponding co-crystallized ligands available in their 3D-structures. The binding interactions of the protein- ligand complexes are shown in the Figure 4(A-J) with their interaction details listed in Table 3. The phytocompound (Clitorin) kaempferol 3-O-(2'', 6''-di-O-rhamnopyranosyl) glucopyranoside (Figure 4A) provided least docking score of -12.574 kcal mol^{-1} and interacted with an active site residue Gln721. This was pursued by (Figure 4B) kaempferol-3-rutinoside (-11.288 kcal mol^{-1}) with an interacting aminoacid residue Gln986, Gln721 and Tyr306.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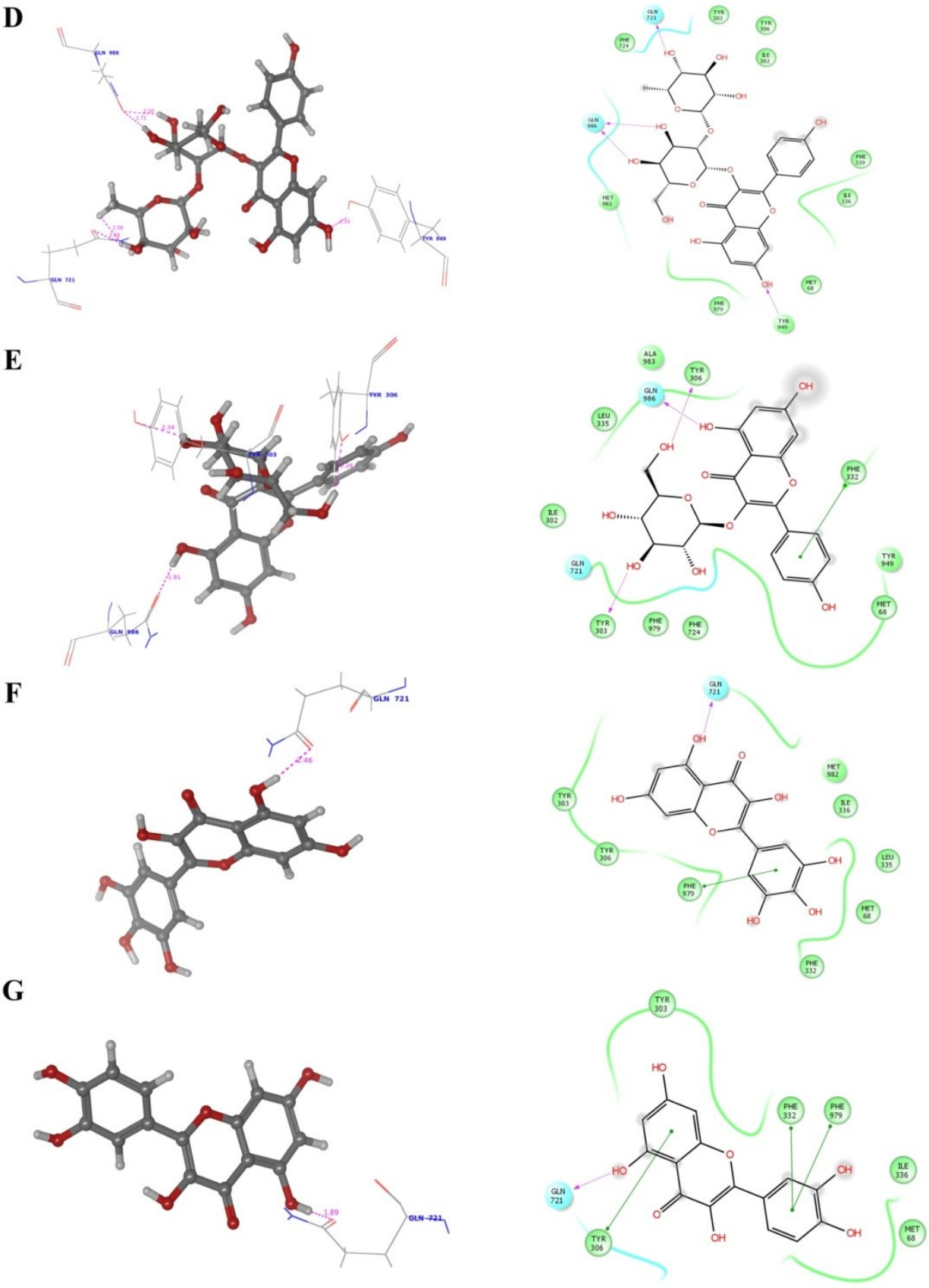
GLN 986
GLN 721
2.45
2.92
1.91
B
GLN 986
GLN 721
2.20
2.00
C
GLN 986
GLN 721
1.73
2.09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GLN 986
GLN 721
TYR 949
E
TYR 306
GLN 986
F
GLN 721
G
GLN 721

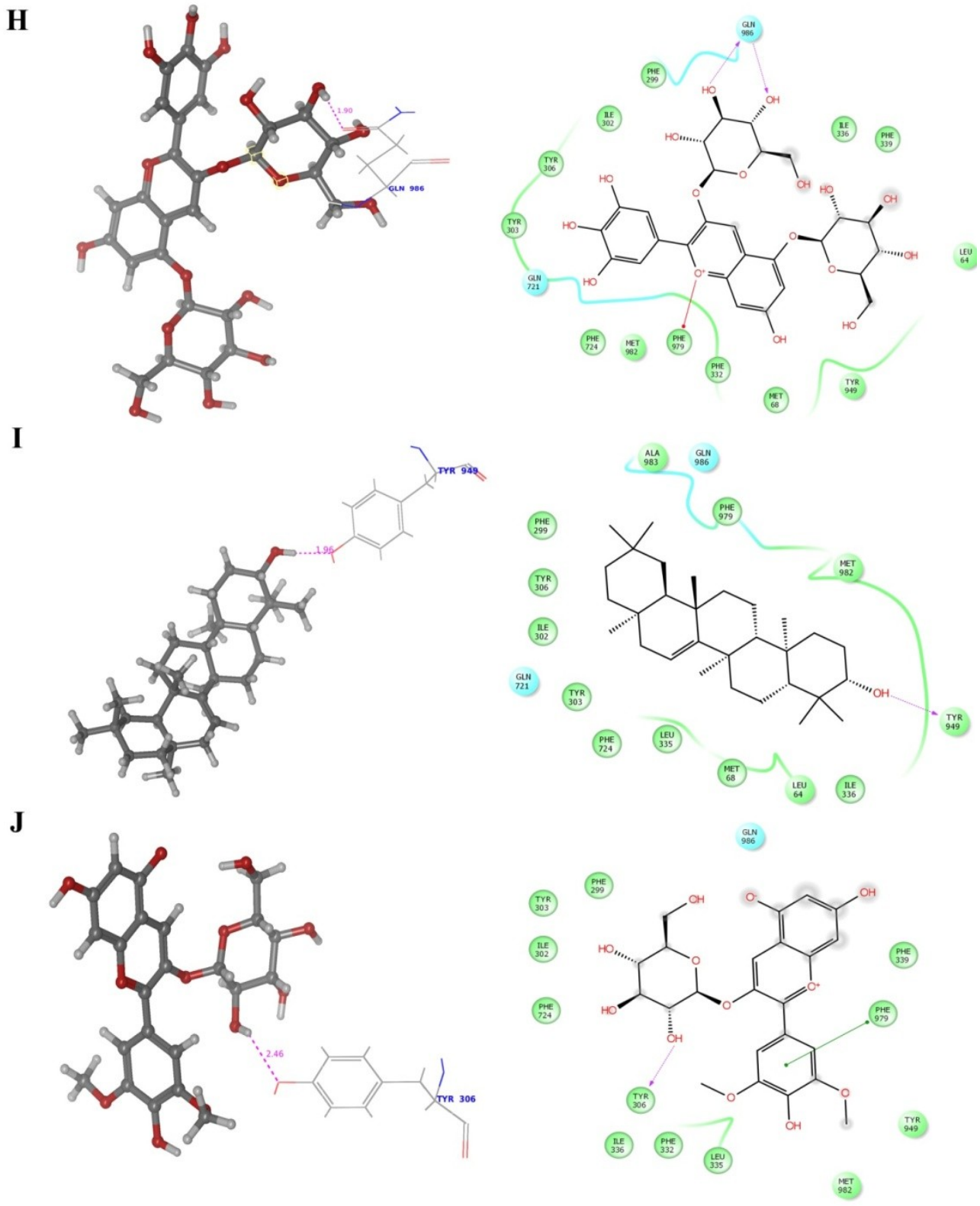

Figure 4. Interacted residues of the Permeability glycoprotein with the standard phytocompounds of *Clitoria ternatea* L. A. (Clitorin) Kaempferol 3-O-(2'',6''-di-O-rhamnopyranosyl) glucopyranoside; B. Kaempferol-3-rutinoside; C. Quercetin 3-neohesperidoside; D. kaempferol-3-neohesperiodoside; E. kaempferol-3-monoglucoside (Astragaline); F. Myricetin; G. Quercetin; H. Delphinidin-3, 5-diglucoside; I. Taraxerol; J. Malvidin-3-O-glucoside (OENIN)

Table 3. Glide (XP) scores and interactions of Phytocompounds of the plant *Clitoria ternatea* L. with the target protein P-gp.

S.No.	Phytocompounds	Protein PDB ID :4M1M			
		Interacting Residues	Bond Length (Å)	No. of H-Bond	XP Glide Score (kcal mol^{-1})
1.	(Clitorin) kaempferol 3-O-(2'',6''-di-Orhamnopyranosyl) glucopyranoside	Gln721 Gln721 Gln721 Gln986	1.91 1.92 2.45 1.58	4	-12.574
2.	kaempferol-3-rutinoside	Tyr306 Gln721 Gln986 Gln986	1.84 2.00 2.20 2.00	4	-11.288
3.	Quercetin 3-neohesperidoside	Ile336 Gln721 Gln986 Gln986	2.67 2.09 1.75 1.73	4	-11.139
4.	kaempferol-3-neohesperiodoside	Gln721 Tyr949 Gln986 Gln986	2.18 2.33 1.71 2.10	4	-10.314
5.	kaempferol-3-monoglucoside (Astragaline)	Tyr303 Tyr306 Gln986	2.34 2.26 1.91	3	-8.736
6.	Myricetin	Gln721	2.46	1	-7.884
7.	Quercetin	Gln721	1.89	1	-7.870
8.	Delphinidin-3,5-diglucoside	Gln986 Gln986	1.90 2.63	2	-7.729
9.	Taraxerol	Tyr949	1.96	1	-6.938
10.	Malvidin-3-0-glucoside(OENIN)	Tyr306	2.46	1	-5.147

Rhodamine 123 accumulation cell assay to analysis the P-gp activity

P-gp activity was determined by measuring intracellular accumulation of rhodamine 123 in hCMEC/D3 cells with the absence or presence of P-gp inhibitors. The uptake of R123 exemplifies a dormancy in the efflux mechanism which is inhibited by the test phytocompounds Kaempferol-3-monoglucoside, Malvidin-3- O -glucoside while the utility of dye infers the P-gp mediated efflux and its regulation. The *in vitro* assay exemplifies a significant increase in the accumulation of Rhodamine 123 percentage when compared to the percentage of the control (Figure 5).

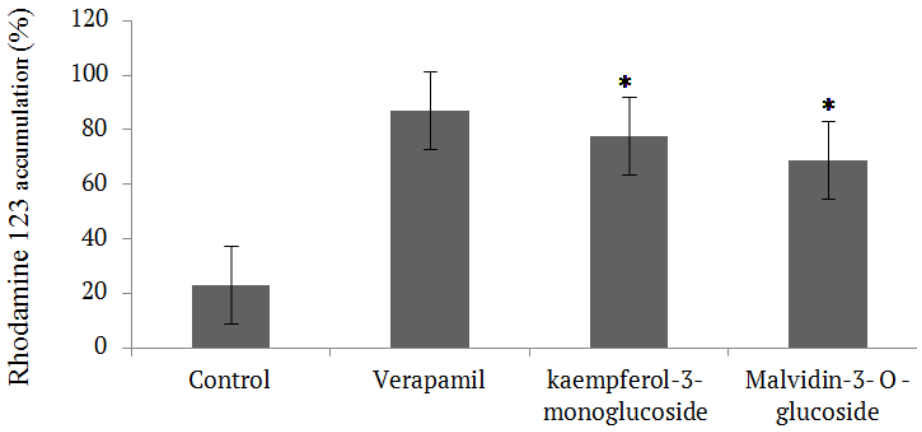

Figure 5. Accumulation of Rhodamine 123 in MDCK –II cell lines in the presence of aqueous plant extract (*Clitoria ternatea* L.) Standard antidepressant (Venlafaxin); Data expressed are the means ±SEM of triplicate measures in comparison to the control (without the presence of drug).

Discussion

The existing prehistoric prose of Ayurveda is curtailed with the mechanist details in terms of understanding the biology and physiology of constituents. Rejuvenated with the technology of advanced science eminence of Ayurveda can intensify globally. Although the attention towards herbal medicine is increasing globally current medicine system claims for active ingredients and principle. It is necessary to discern the therapeutic potential of the phytocompounds and *in silico* analysis paves way to explore drug efficiency. Hence this study emphasizes *Clitoria ternatea* L. as an antidepressant by an in vivo assay with its aqueous leaf extract. Further the work is coordinated with an *in silico* analysis to invigorate the specific phytocompounds that can inhibit the efflux protein P-gp to surpass blood brain barrier. Thus, this work aids in furnishing the active compounds of the ayurvedic herb that can combat depression and establish as an effective brain drug. The aqueous leaf extract exhibited a significant dose dependent (50-100mg kg^{-1}) decrease in the immobility, time when compared with the control and did not exhibit a great extent of discrepancy. The aqueous extract was opted rather from the organic solvents in order to reduce cytotoxicity. The oral administration of leaf extract has enhanced the potential of antidepressants effectively to brain. This offers a hopeful stratagem to overcome the adverse effects caused by the standard drugs and can extend secure recovery against mental illness. The ayurvedic medicinal plant *Clitoria ternatea* L. has attested its prophecy as a natural psychotherapeutic agent that can confront depression.

The behavioral tests are modeled according to the environmental stress created to the animals (Detke, Rickels, & Lucki, 1995; Dhingra & Sharma, 2006). In the forced swim model the animals are restricted to swim in a space where escape is not possible and finally they reach a state of helpless despair syndrome which is claim to comprehend similar circumstances of human depression (Lucki, 1997; Dhingra & Valecha, 2007). The Tail suspension test is considered as a sensitive technique that has been studied in various antidepressant arrays such as Monoamine Oxidase Inhibitors (MAOIs), Selective serotonin reuptake inhibitors (SNRIs) and (TCAs) Tricyclic antidepressants (Thierry, Stéru, Simon, & Porsolt, 1986). The results show that leaf extracts of *Clitoria ternatea* L. can decrease immobility time in both forced swim and tail suspension test. It is instituted that the plant can produce antidepressant like activity at a dose of 100 mg kg^{-1} body weight of animal exposed to a treatment phase of one week. The decrease in the immobility time is accompanied with the increase in the behavior patterns such as swimming and climbing. Oxidative damage induced to brain implicates reduction in brain energy metabolism which causes more micro vascular constriction and reduction in blood flow that leads to microgliosis. The degeneration in the glial cells persuades cytokines, which are responsible for mononuclear leukocyte cell accumulation that results in cellular edema (Sofroniew & Vinters, 2010). Polymorphic infiltration generally, occurs due to the destruction in the epithelial cell linings which is due to the stress induced to the mice. The detrimental effects of the lymphocytic filtration deplete the fat cells in the tissue that lead to destruction and necrosis of nucleus. Eventually, this can cause a pathological condition called as hyperplasia (Hamby & Sofroniew, 2010). Subsequently, Figure 3B depicts the normal mice which has not been induced to depression and the treated mice with the plant extract. Less and mild changes are observed in the cerebral cortex regions of the treated mice with the plant extract, which exemplifies the significance of the plant *Clitoria ternatea* L. as an efficient brain drug that has the ability to reverse the degenerative changes, gliosis and cellular edema and can reinstates psychological illness.

The *in silico* study investigated miscellany in the amino acid residues such as Tyr306, Gln986, and Ile336 which are distinct from the amino acids of the active site. The active sites associated with the QZ59-RRR ligand consists of amino acid residues, such as Met88, Phe332, Ile338, Phe338, Gln721, Tyr949, Phe724, Phe974, Val978, Tyr303, Phe728, Ser975 and Leu335[28] .Most of the residues in proteins active site are hydrophobic and are involved in a sturdy hydrophobic interaction. The ligands in the study exhibited a good interaction with the target protein's active site (Gln721) attesting a strong hydrophobic interaction that prevail the binding score of a western folk antidepressant phytocompounds Pseudohypericin and hypericin (Dhayabaran & Margret, 2016).

Though, (Clitorin) kaempferol 3-O-(2'',6''-di-O-rhamnopyranosyl) glucopyranoside exhibited a least docking scores, the molecular weight of this phytocompound is comparatively high(740.668 g mol^{-1}) and generally, a drug acquires molecular weight of less than 500 g mol^{-1}. However, to substitute this kaempferol-3-monoglucoside (Astragaline) and Malvidin-3-0-glucoside (OENIN) blemishes a convincing docking score of (-8.736 and -5.147 kcal mol^{-1}) with an ideal molecular weight of 448.3 and 426.7 g mol^{-1}. Further, the

pharmacological studies substantiated (Table 2) that Malvidin-3- O -glucoside (OENIN) confers a better pharmacokinetic property with a high BBB permeation, normal polar surface area (0.0 to 150.0) values and restricting into the optimistic molecular weight. The molecular docking study as mentioned above has a good interaction score that characterizes, the phytocompound as a promising ligand that passed through the pre-screening phase on pharmacological properties (ADMET) which validates the efficiency of a drug (Banik, 2004; Butina, Segall, & Frankcombe, 2002) .Hence the *in silico* study endorses the phytocompound to be a more effective brain drug that can pass through the blood brain barrier.

Rhodamine 123, a member of the rhodamine family of flurone dyes, has been used to examine membrane transport potential of drugs deferring the capacity of efflux proteins (Le Vee, Jouan, Stieger, Lecureur, & Fardel, 2015).The presence of standard P-gp inhibitory drug (Verapamil) exposed the drug to a greater rate of accumulation which relatively analysed the permeability potential of drug Kaempferol-3-monoglucoside and Malvidin-3- O –glucoside . The P-gp mediated efflux was regulated and with an increase accumulation of dye when compared to cells that lack P-gp inhibitor. Fluorescent tracer dyes represent an important class of sub-cellular probes and allow the examination of cellular processes with a simple mechanism. Mitochondrial membrane potential is a significant feature that contributes to the accumulation and utilization of the dye (Kim, Cooper, Hayes, & Spangrude, 1998). Generally, drugs interacting with mitochondria decrease its transmembrane potential and allow a distinct difference of the P-gp (Shityakov & Förster, 2014) inhibiting drugs which allows a profound accumulation of R 123 through its inhibiting efflux. This discrete the ability of a drug to surpass efflux mechanism from a drug that lacks this potential (Varga, Ferdinandy, Liaudet, & Pacher, 2015).Thus from this assay it is apparent that the phytocompounds has the probability to restore the efflux mechanism and reach the targeted site.

Several studies have endorsed *Clitoria ternatea* L. as a potential neuroprotectant through various animal models but, the present work substantiates its dual role in acting against depression and bypassing the blood brain barrier. Conversely, the efficacies of specific phytocompounds are validated in this work with both *in silico* and *in vitro* assays against the efflux protein P-glycoprotein. Apart from anxiety and stress related psychotic disturbances, neuropathological issues also require an immediate antidote. Seizure related behavioural models have been reported (Aragao et al., 2016) with mice which can be further standardized using the phytocompounds as therapeutic agents. Hence various drug parametric and pharmacological assays are required.

Conclusion

The current study aggravates the importance of the plant *Clitoria ternatea* L. an ancient *Ayurvedic* medicinal herb which can combat depression and serve as an efficient brain drug that can pass through the blood brain barrier. The effectiveness of these potent phytocompounds as a synergetic effect is endorsed by performing clinical studies which elevates the incredible competence of *Ayurvedic* medicine on parity with western medicine. This research thus provides the right thrust in including the plant as a therapeutic agent confronting depression thereby bestowing effective herbal remedy with lessen adverse effects. The aqueous leaf extract exhibited a significant decrease in the immobility, time when compared with control. The accomplished antidepressant activity of the plant persuaded a futuristic effort to surpass the blood brain barrier by inhibiting P-gp. The docking complex of the phytocompounds [kaempferol-3-monoglucoside (Astragaline) and Malvidin-3- O -glucoside (OENIN) kaempferol-3-monoglucoside (Astragaline) and Malvidin-3- O -glucoside (OENIN)] persuaded a futuristic effort as lead molecules to surpass the blood brain barrier by inhibiting P-gp which was substantiated with the rhodamine 123 transport assays. The ADMET studies furnished eight potential phytocompounds with good pharmacological properties which can be persuaded with IC_{50} values and authenticated further with higher animal models

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