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EFFECT OF *HYLOCEREUS UNDATUS* FRUIT EXTRACT AGAINST DENGUE ASSOCIATED THROMBOCYTOPENIA IN WISTAR RATS AND MOLECULAR DOCKING STUDIES

Pharmacology

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ABSTRACT

OBJECTIVE: To investigate the effect of *Hylocereus undatus* fruit extract against Dengue associated thrombocytopenia in Wistar rats and molecular docking studies.

MATERIALS AND METHODS: (a) The aqueous extract of dried fruit of *Hylocereus undatus* was extracted using cold maceration process and the extract were divided into different concentration such as 200mg/kg and 400mg/kg and *In vivo* studies were performed to investigate bleeding time, clotting time and platelet increasing activity and compared with the standard drug prednisolone (0.5mg/kg). (b) Phytochemical studies were evaluated using HPLC technique. (c)Molecular docking studies involving the interaction between phytochemicals of *Hylocereus undatus* and dengue viral proteins.

RESULTS AND DISCUSSION: The results revealed that fourteen-day administration of 200 mg/kg and 400mg/kg of *Hylocereus undatus* showed increased of platelet count in dose dependant manner, 400mg had significantly increased platelet counts (11.55±0.36) x10^5/µl when compared to control group. Similarly, the bleeding time was decreased and the clotting time was increased in ethanol-induced thrombocytopenic rats.

MOLECULAR DOCKING: Phytochemicals found in *Hylocereus undatus*, specifically quercetin, β amyrin, gallic acid γ sitosterol, kaempferol, were used for docking study using AUTODOCK software applications. The dengue protease (2FOM) and dengue methyl transferase (2P40) have been selected as targets for binding assays.

CONCLUSION: According to the study, it was concluded that the *Hylocereus undatus* is a potential therapy against thrombocytopenia in dengue as it possesses a significant platelet increasing activities. Out of the phytochemicals, quercetin was found to exhibit the strongest binding with dengue targets. Hence, this study indicates that the *Hylocereus undatus* is effective against dengue virus.

KEYWORDS

Hylocereus undatus, HPLC technique, molecular docking, Thrombocytopenia.

1.INTRODUCTION

Dengue, a delibilating viral disease is caused by RNA virus named Dengue fever virus [denv][1], belonging to the family *Flaviviridae*, of genus Flavivirus. It is disseminated by *Aedes mosquito*, particularly *Aedes aegypti*[2]. It reproduces inside the cells. The white blood cells respond by produces signaling proteins - cytokines and interferons. Fluid from the blood stream leaks through small blood vessels due to capillary permeability[3]. As a result, less blood circulates in the blood vessels, and the blood pressure becomes so low that it cannot supply sufficient blood to vital organs, furthermore, dysfunction of the bone marrow due to the infection of the stromal cells leads to reduced number of platelets. Dengue is provoked from four serotypes-DENV-1, DENV-2, DENV-3 and DENV-4. The dengue virus genome accommodates about 11,000 nucleotide bases which codes for 3 varieties of protein molecules- C, prM and E which forms the virus particle including 7 other structural protein molecules [NS1,NS2a,NS2b,NS3,NS4a,NS5a] accountable for viral replication[4]. Since there is no effective treatment against dengue, people are now focusing mainly on the herbal medicines as a trusted approach for its no side effects. Numerous nutraceutical as well as herbal medicines[5][6] combination are upcoming formulation in market, proving their efficiency in providing curative treatment. Due to the increased thrombocytopenic effect, there leads to Dengue Haemmorhagic fever[7][8][9]. The present investigation was therefore aimed at the effect of Hylocereus undatus fruit extract against the thrombocytopenia induced Wistar rats and molecular docking studies[10][11]

2.MATERIALS AND METHODS

2.1 Plant Materials:

The fresh fruit was collected and authentified by SIDDHA CENTRAL RESEARCH INSTITUTE, CHENNAI. With reference no 115.05021901.Then dried at room temperature and was made into coarse powder. The powder was extracted by cold maceration technique using water as a solvent and the yield was stored at room temperature[13][14].

2.2 Preparation of the extract:

Cold maceration is the extraction process that consist off maintaining contact between the plant and the solvent for a period of time. It is conducted at room temperature The soul purpose of such extraction procedure for crude drugs is to obtain the therapeutically desirable portion and eliminate the inert material with the selective solvent known as menstrum It plays a decisive role in the qualitative and quantitative composition of the extracts. These extracts are utilized for the isolation and characterization of therapeutically active chemical constituents used in modern medicine.

2.3 Preliminary Phytochemical Screening

The chemical tests for various Phyto constituents in the aqueous extracts on H.undatus,,were carried out and the results were recorded.

2.4 Animals:

The animals Wistar rats (160g-180g) were procured from ANIMAL ETHICAL COMMITTEE IN K.K.COLLEGE OF PHARMACY with approval no:KKCP/2019/07 .The study was approved by INSTITUTION OF ANIMAL ETHICAL COMMITTEE and the work was carried out as per CPCSEA guidelines, New Delhi.

2.4.1.Acute toxicity study (OECD guideliness 423)

12 Female *Wistar Albino* rats weighing about (150-250 gm,were taken in the study.The acute toxicity is carried out as per the guidelines set by Organisation For Economic Cooperation and Development (OECD) revised draft guidelines 423 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

It is a stepwise procedure with three animals of a single sex(with female) per step..Depending on the mortality and morbidity of the animals a few step may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data.The method uses defined doses (5,50,300,2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system. The dose was administered to the rats which were fasted overnight with water ad libitum and observed for signs of toxicity.

2.4.2 Anti-thrombocypenic activity

The animals were given ethanol [0.2ml] by intraperitoneal injection to induce thrombocytopenia and after 24 hrs[15][16], blood samples were collected by retro orbital puncture to determine the decrease in platelet count. Subsequently the groupings were proceeded.

SNO	GROUP	TREATMENT	NO OF ANIMALS
1	Group1	Vehicle control(0.1ml distilled water)	6(3male+3 female)
2	Group2	Standard(0.2ml Anagrelide)	6(3male +3 female)
3	Group3	Low dose(200mg/kg of H. undatus	6(3 male+3female)
4	Group4	High dose(400mg/kg of H. undatus)	6(3male+3 female)

The animals will be administered with the fruit extract and the standard drug for about 14days[17][18].Finally, at the end of the 14th day, the blood sample will be collected by retero-orbital puncture to find out the increase in the platelet count.

2.4.3. Statistical Analysis

The results are presented as mean ± SEM. "One- way ANOVA with Dunnets post was performed using Graph pad Prism 3.00 for windows. P<0.01 were considered as significance

2.4 Molecular Docking[19][20]

Docking studies were carried out to find the inhibitory activity of the compounds obtained from HPLC analysis of aqueous extract of Hylocereus undatus. Among the 3 compounds, Quercetin, Kaemferol, Isorhamnetin ,quercetin showed best docking result based on the binding energy. The study is carried out using AUTO DOCK 4.2 SOFTWARE. **Dengue protease (2F0M) and dengue methyl transferase (2P40)** were selected as target for binding assay.

3.RESULTS AND DISCUSSION

3.1 Preliminary Phyto Chemical Studies

The results of preliminary phytochemical analysis of the aqueous extract of Hylocereus undatus shows the presence of Flavanoids , Glycosides ,Amino acids ,Tannins , Steroids , Phenols and Saponins

3.2Acute toxicity study (OECD GUIDELINESS 423)

The animals were treated with aqueous extract of Hylocereus undatus did not change the autonomic or behavioural responses in rat. There is no mortality upto 2000mg/kg body weight.

S.no	Parameters	Result
1	Tremors	Absent
2	Convulsion	Absent
3	Straub reaction	Absent
4	Pilo erection	Absent
5	Loss of lighting effects	Absent
6	Sedation	Absent
7	Muscle relaxation	Absent
8	Hypnosis	Absent
9	Analgesia	Absent
10	Ptosis	Absent
11	Lacrimation	Absent
13	Diarrhoea	Absent
14	Change in skin colour	No change

Acute toxicity studies of *Hylocereus undatus* fruit extract in Wistar rats

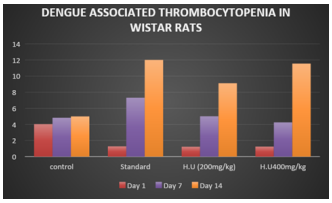
3.3 PHARMACOLOGICAL STUDY

Anti-thrombocytopenic activity

The anti-thrombocytopenic activity of HU was analysed by invivo method by using Wistar Albino rats of both sex. The activity was performed and the increase in the platelet count were noted.

Grouping	Day1	Day7	Day14
Control	4.03±0.21	4.82±0.21	5.0±0.64
Std(Anagrelide5mg/kg)	1.28±0.24	7.32±1.04***	12.42±0.12***
H.U (200mg/kg)	1.22±1.20	5.01±0.24***	9.12±1.24***
H.U400mg/kg	1.24±0.12	4.26±0.12***	11.55±0.36***

Anti-thrombocytopenic activity of *Hylocereus undatus* fruit extract in Wistar rats

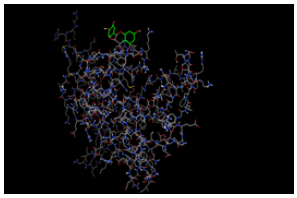


Graphical representation for the anti-thrombocypenic activity of Hylocereus undatus aqueous extract compared to the standard drug Anagrelide

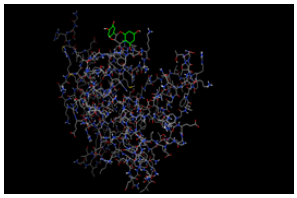
- The results revealed that fourteen-day administration of 200 mg/kg and 400mg/kg of *Hylocereus undatus* showed increase in platelet count in dose dependant manner, 400mg had significantly increased platelet counts (11.55±0.36) x10^5/µl when compared to control group.

3.4 Molecular Docking Results

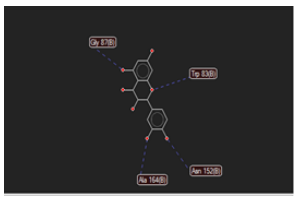
Dengue Protease Enzyme



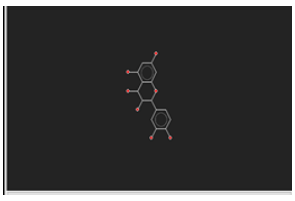
A.Ligand and protein binding



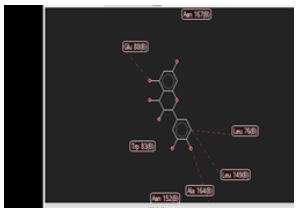
B. Ligand interaction



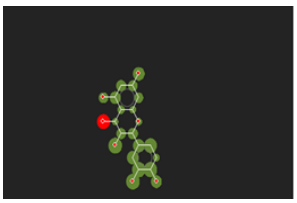
C.Hydrogen bonds



D. Electrostatic interaction

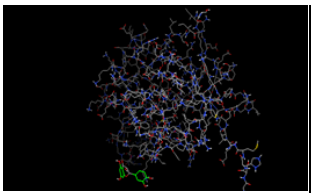


e. steric interaction

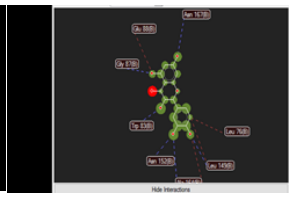


F. interaction overlay

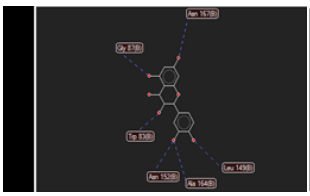
Dengue Methyl Transferase



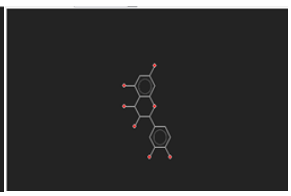
A.Ligand and protein binding



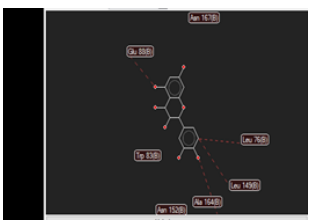
B. Ligand interaction



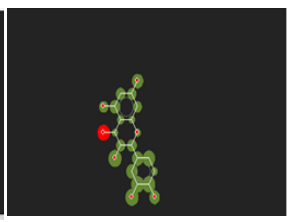
C.Hydrogen bonds



D. Electrostatic interaction



e. steric interaction



F. interaction overlay

Binding Energy
Using Dengue Transferase Enzyme

RANK	BINDING ENERGY
1	-10.29
2	-10.22
3	-9.82

4	-9.21
5	-8.70
6	-8.42
7	-7.95

Using Dengue Protease Enzyme

RANK	BINDING ENERGY
1	-10.29
2	-10.22
3	-9.48
4	-8.68
5	-8.70
6	-8.45
7	-7.95

DISCUSSION

The phytochenical analysis of HU has demonstrated the presence of alkaloids, glycosides, saponins,phytosterols, phenolic compounds and flavanoids. Phenolics and flavanoids are well known compounds with platelet increasing potential. Animal studies conducted using Wistar Albino rats established that the oral administration of HU fruit increases the platelet count. HU has significantly increased the platelet count in rats treated with ethanol to induce thrombocytopenia.. In Molecular Docking, the Protein Data Bank ID obtained using AUTO DOCK -4 obtained the binding energies, of which the lowest binding energies of Dengue Protease Enzyme were found to be -10.29, -10.22, -9.82 and Dengue Methyl Transferase with -10.29, -10.22, -9.48 considered as significant activity.

CONCLUSION

H.undatus fruit extract ,can be used as a medicine to boost thrombocytopenia, when these have been suppressed by the disease. However, More work is needed to isolate and to identify biologically active ingredients of *H.undatus* fruit that are responsible for these effects Further, well-controlled double-blind clinical trials are required to re evaluate the efficacious and the side effects in order to establish its place in clinical applications for its wide utility in the society

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