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Evaluation of the Effect of Platelet Increase in Trichosanthes Cucumerina by using Albino Rats

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Abstract: *The objective of the present study was to evaluate the effect of Trichosanthes Cucumerina in increasing the platelet count. Trichosanthes Cucumerina (Cucurbitaceae) is a traditional medicinal plant known as snake guard. Snake gourd is an annual climber and it's commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to it is good nutritional value. The fruit is a good source of Vitamin A, Vitamin B and Vitamin C. It improves the appetite and acts as a tonic and stomachic and cures biliousness. This is one of the most genetically diverse groups of food plant in the plant kingdom and every part of this plant is used to treat various diseases. It is used in the treatment of head ache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhea, hematuria and skin allergy. T. curcumineriais used as an abortifacient, vermifuge, stomachic, refrigerant, purgative, malaria, laxative, hydragogue, hem agglutinant, emetic, cathartic, bronchitis and anthelmintic. study was undertaken to evaluate the possible increasing platelet count effect of Trichosanthes Cucumerinafresh juice extract (TCJE) using Cyclophosphamide induced thrombocytopenia. Group-I (control) received normal saline (1ml/100gm), Group-II receiving Cyclophosphamide (200mg/kg), Group III & IV received Cyclophosphamide and TCJE in doses of 200 mg, 200 & 400 mg/kg orally (P.O.) respectively. Blood samples were collected after 60 minutes of dose administration and TCJE produced significant increase in platelet count like effect at dose of 200 & 400 mg/kg administered for 7 & 14 consecutive days as indicated by increasing blood parameters like Hemoglobin, total leucocytes count, polymorphs, lymphocytes, eosinophils, monocytes, platelet count and red blood cells count of Rat in TST & FST ($P<0.05$). The efficacy of ZJPE at 400mg/kg was found to be comparably more.*

Keywords: *Trichosanthes Cucumerina, Cyclophosphamide, Hemoglobin, total leucocytes count, polymorphs, lymphocytes, eosinophils, monocytes, platelet count and red blood cells*

1. INTRODUCTION

Blood platelets also known as thrombocytes[1, 2] play an important role in your body. These cell fragments are natural source of growth and play basic role in process of hemostasis (preventing bleeding from damaged blood vessel) as well. Normal platelet count in a micro liter of human blood is 150000 to 450000. Slight decrease in the platelet count does not contribute to the disease but when it is below 50,000 permicroliter, thrombocytopenia[3, 4]occurs. Low platelet count in dengue fever can be very dangerous. The reasons for low platelet count in dengue fever are as follows. Dengue virus[5], the main cause of dengue fever induces bone marrow suppression. Since bone marrow is the manufacturing center of blood cells its suppression causes deficiency of blood cells leading to low platelet count. Anaemia[6] and spontaneous severe bleeding are the other resultant factors of bone marrow suppression. Studies suggest that dengue virus can even bind to platelets of human blood in the presence of virus-specific antibody. When vascular endothelial cell that are infected with dengue virus gets combined with platelets they tend to destroy platelets[7]. This is one of the major causes of low platelet count in dengue fever. Even the antibodies that are produced after infection of dengue virus can contribute in destruction of platelets, thus lowering the platelet count[8].Platelet counts are a lab measure of the concentration of platelets in the blood. A normal platelet count is around 140,000 to 450,000 platelets in every microliter of blood. When the number of platelets is low, this concentration goes down. Low platelet count in dengue fever may lead to life-threatening condition known as hemorrhagic dengue fever that is categorized by spontaneous

bleeding tendency and shock."Many viral infections, which are not at all dangerous, can also reflect a low platelet count. Several pathology labs in the city have reported a reduction in platelet count in blood samples. There is no vaccine available for dengue so far. "Instead of going for platelet count every day, newer tests such as NS1 antigen should be done on priority in the first week of illness, if there is any suspicion of dengue. All viral illnesses represent themselves in the same fashion as in dengue". Patients are usually advised a platelet count test since a low platelet count accompanied by other symptoms such as fever, headache, muscle and joint pains usually indicate dengue. When the blood reports show a low platelet count, many conclude that they may have contracted dengue and seek hospitalization[9, 10].Snake gourd is scientifically called as Tricosanthes cucumerina is a well-known plant, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to it is various pharmacological activities like antidiabetic, anti-platelet, hepatoprotective, cytotoxic, anti-inflammatory, larvicidal effects[11-13].

2. MATERIALS AND METHODS

2.1. Plant Extraction

Six ripe fruits of Trichosanthes cucumerina were gotten from chebrolu, a local town in Guntur, Andhra Pradesh. The Fruit were obtained and, washed in clean water; sun dried for three days, the coat peeled off, 40g fruit were weighed, grinded into fine juice and finally socked in 100ml of water. The solution was filtered after 48 hours while the filtrate was concentrated using the rotary evaporator; volume of filtrate obtained =30.6ml, weight of residue left =32g; weight of the fruit dissolved in the filtrate= 8g; volume of filtrate after evaporating = 2.5ml.

Therefore; extracts concentration= mass/volume=8/2.= 3.2g/ml

2.2. Phytochemical Testing

2.2.1.Tests Performed for the Presence of Phytoconstituents

2.2.1.1. Tests for Alkaloids

Dragendorff's test: To 1ml of each of the sampale solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids. .

2.2.1.2. Tests for Glycosides

Raymond's test: Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet color

Legal's test: When the test samples were treated with pyridine and sodium nitroprusside solution blood red colorappears.

2.2.1.3. Tests for Tannins and Phenolic Compounds

Ferric chloride test: When few drops of ferric chloride were added to sample solution a blackish precipitate appears. .

Gelatin test: When gelatin and water were added to test samples resulted the formation of white precipitate.

Lead acetate: Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It results in the formation of reddish brown bulky precipitate.

2.2.1.4. Tests for Flavonoids

Zinc Hydrochloride reduction test: To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red color.

Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

2.2.1.5. Tests for Sterols

Libermann-Buchard test: When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

2.2.1.6. *Tests for Fats and Oils*

1. Stain test: Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of oils.

2.2.1.7. Tests for Lignin

Labat test: When Gallic acid is added to the test sample, it results in the formation of olive green color.

Furfur aldehyde test: When furfur aldehyde is added to the test sample a red color appears indicating the presence of lignin.

2.2.1.8. Tests for Quinones

Alcoholic KOH test: When alcoholic KOH was added to the test samples red to blue color appears reacting positively for quinones.

2.2.1.9. Tests for Saponins

Foam test: 5 ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicates the presence of saponins.

3. ANIMALS

Albino rats weighing around 200g – 300g of either sex were obtained from Nirmala College of pharmacy, mangalagiri. Animals had free access to food and water and maintained under standard laboratory conditions with a natural light and dark cycle. The animals were acclimatized for at least five days before behavioral experiments. Experiments were carried out between 9.00 and 15.00 hrs. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

3.1. Acute Toxicity Studies

Aqueous extract of T.cucurmina (was studied for acute oral toxicity as per revised OECD (2002) guidelines No. 423. Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 200-400 mg/kg doses of extract were used.

3.2. Platelet increase Activity

3.2.1. Experimental Design for Platelet increase activity

The animals were selected randomly for each experiment and divided into 6 equal groups. Drugs (Control, TCJE, Cyclophosphamide) administered orally (P.O.) for 7&14 successive days as depicted.

Group-I –Control (Normal saline)

Group-II - Cyclophosphamide (200mg/kg)

Group-III - Cyclophosphamide (200mg/kg) + TCJE (200mg/kg)

Group-IV - Cyclophosphamide (200mg/kg) + TCJE (400mg/kg)

60 minutes after the administration of doses, the blood samples are withdrawn from retro orbital route and following blood parameters like Hemoglobin, total leucocytes count, polymorphs, lymphocytes, eosinophils, monocytes, platelet count and red blood cells were investigated.

3.3. Statistical Analysis

All the values were expressed as Mean ± S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test, P<0.05 was considered significant.

4. RESULTS

4.1. Preliminary Phytochemical Screening

Table1. *Phytochemical Analysis of Trichosanthes cucumerina*

Test for Phytoconstituents	Aqueous Extract
Test for Alkaloids003A	
Dragendroff's Test	+
Mayer's Test	+
Wagner's Test	+
Hager's Test	+
Tanicacid Test	+
Test for glycosides	
Raymond's test	-
Legal's test	-
Bromine water test	+
Kellarkiliani test	+
Molish test	+
Test for tannins	
Gelatin test	+
Lead acetate test	+
Shinoda's test	+
Test for flavonoids	
Zn-Hcl test	+
Shinoida's test	+
Alkane reagent test	+
Test for sterols	
LibermannBurchard test	+
Salkoswki test	+
Test for Fats and Oils	
Stanin test	-
Saponification test	-
Tests for quinones	
Alcoholic KOH	-
Foam test	-

On preliminary phytochemical analysis of TCJE showed the presence of sterols, flavonoids, glycosides, alkaloids, carbohydrates and proteins.

4.2. Acute Toxicity Studies

Tricosanthes cucumerina had not shown neither any behavioral changes, nor mortality at dose 2000 mg/kg.

4.3. Platelet Increase activity

In normal control control rats the parameters are recorded, those are Hemoglobin (12.0±0.9gm/dl), leucocytes (6.900±1.500cells/cumm), polymorphs (62±1.0%), lymphocytes (35±0.9%), eosinophil (02±001%), monocytes (0.1±0001%), platelet count (224000±3000lakh/column), red blood cells count (4.22±0.8millions/cumm). When compared to control grouped animals, cyclophosphamide induced group shown result of the parameters like Hemoglobin (11.4±0.8gm/dl) was decreased, total leucocytes count (7,100±0.900cells/cumm), polymorphs (67±0.9%), are significantly increased, lymphocytes (30±1.0 and 35±1%), eosinophil (02±001%), monocytes (0.1±001%), red blood cells count (4.42±0.15millions/cumm) values are not changed and platelet count (98,000±2500lakh/cumm) is significantly decreased. The group

3 and 4 had produced Hemoglobin (12±1.0 and 12.3±0.1gm/dl), total leucocytes count (7,100±0.50 and 7,200±0.800 and cells/cumm), polymorphs (65±0.5and 62±1%), are significantly increased, lymphocytes (30±1.0%), eosinophil (02±001%), monocytes (0.1±001 and 0.2±001%), red blood cells count(4.42±0.15 and 4.40±0.25 million/cumm) values are not changed and platelet count (98,000±2500 and 2, 12,000±1,000 lakh/cumm) is significantly decreased.

Table3. *Effect of trichosanthes cucumeriuna by increasing platelet count was shown by these parameters*

Groups	Animals	Hemoglobin	Leucocytes	Polymorphs	Lymphocytes	Esinophil	Monocytes	Platelet count	RBC
NormalControl	H	12.0	6.900	62	35	02	01	2240000	4.22
	B	12.85	7.100	66	42	01	01	250000	3.98
	T	13.21	7.600	78	45	05	02	290000	4.29
	HB	14.5	8.000	80	39	06	02	215000	5.26
	HT	11.65	6.500	65	36	04	01	216000	5.92
	BT	11.98	7.200	72	43	03	01	240000	6.0
	Mean±SEM	**12.6±0.43**	**7.216±0.81**	**70.5±1.94**	**40±0.1**	**35±0.65**	**1.5±0.1**	**239000±100**	**4.94±0.46**
Cyclophosphamide (200mg/kg)	H	11.4	7.100	67	30	02	01	98000	4.22
	B	11.8	7.500	68.6	32	01	02	96000	4.12
	T	12.6	8.500	70.3	39	03	02	85000	4.98
	HB	13.6	9.000	65.4	40	05	02	86000	4.75
	HT	14.3	6.500	68.4	38	06	01	76000	5.26
	BT	15.,0	7.800	65.9	36	05	01	80000	6.0
	Mean±SEM	**13.08±0.43**	**7.733±0.81**	**67.6±1.94**	**36±0.1**	**3.5±0.65**	**1.5**	**86000±100**	**4.88±0.46**
Cyclophosphamide and extract juice first dose(200mg/kg)	H	12.7	8.600	70	25	03	02	197000	3.92
	B	12.9	9000	70	28	03	01	210000	3.65
	T	13.5	9.200	69	30	04	01	215000	4.21
	HB	14.3	9.800	68	35	05	02	225000	4.95
	HT	15.0	8.400	65	38	02	02	265000	5.20
	BT	13.8	7.900	62	29	02	02	225000	5.20
	Mean±SEM	**13.68±o.43**	**8.816±0.81**	**67.3±1.94**	**30.83±0.1**	**3±0.65**	**1.66±0.1**	**222000±100**	**4.48±0.46**
Cyclophosphamide and extract juice second dose(400 mg/kg)	H	11.3	7.800	60	35	03	02	212000	4.18
	B	11.9	7.900	62	36	03	02	250000	4.52
	T	12.5	8.200	68	38	04	02	269000	4.96
	HB	13.0	8.500	70	39	05	01	298000	5.25
	HT	13.5	8.600	69	40	06	01	315000	5.9
	BT	12.6	7.700	63	36	02	01	242000	4.2
	Mean±SEM	**12.46±o.43**	**8.116±0.81**	**65.3±1.94**	**37.3±o.1**	**3.83±0.65**	**1.33±0.1**	**314000±100**	**4.87±0.46**

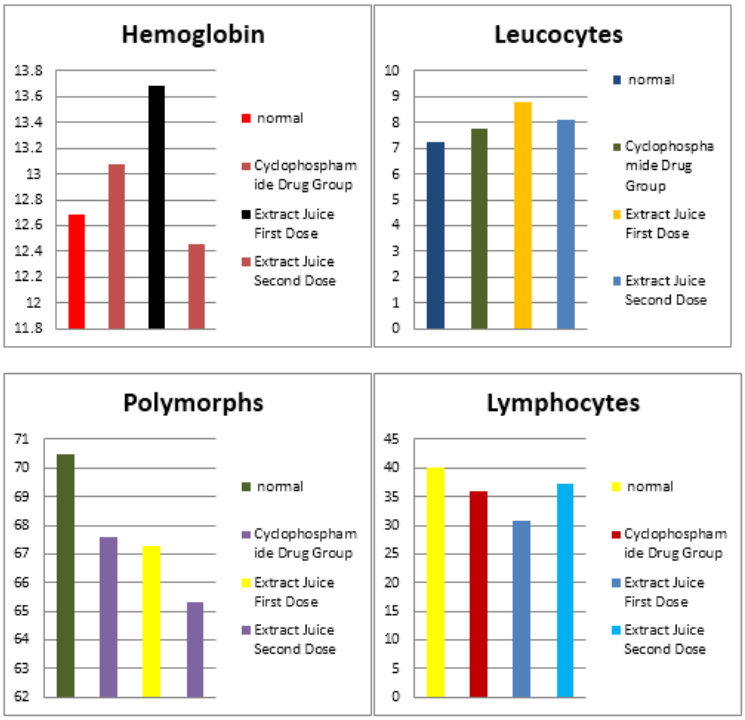

Fig. *Bar Diagrams for Hemoglobin, Leucocytes, Polymorphs and Lymphocytes*

5. DISCUSSION

Platelets, also called thrombocytes (thromb- + -cyte, "blood clot cell"), are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries. Platelets have no cell nucleus: they are fragments of cytoplasm that are derived from the megakaryocytes of the bone marrow, and then enter the circulation. These inactivated platelets are biconvex discoid (lens-shaped) structures, 2–3 μm in greatest diameter. Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells. Normal platelets can respond to an abnormality on the vessel wall rather than to hemorrhage, resulting in inappropriate platelet adhesion/activation and thrombosis: the formation of a clot within an intact vessel. This type of thrombosis arises by mechanisms different than those of a normal clot: namely, extending the fibrin clot of venous thrombosis; extending an unstable or ruptured arterial plaque, causing arterial thrombosis; and microcirculatory thrombosis. An arterial thrombus may partially obstruct blood flow, causing downstream ischemia, or may completely obstruct it, causing downstream tissue death. In normal group the platelet shows its normal platelet activity. When compared to toxicants control, Trichosanthes cucumerina was treated with animals have shown a significant reduction, in these platelet activation in trichosanthes cucumerina was increasing the platelet count, lymphocytes and polymorphs are totally increased when compared the normal and cyclophosphamide groups. The cyclophosphamide studies was determined the low Platelet count and lymphocytes was totally decreased condition when compared to normal groups. When compared to toxicants control, Trichosanthes cucumerina was treated with animals have shown a significant reduction. In these platelet activation in trichosanthes cucumerina was increasing the platelet count, lymphocytes and polymorphs are totally increased when compared the normal and cyclophosphamide groups. When compared to Normal control trichosanthes cucumerina was treated with these animals have shown a dose dependent platelet activity as seen with a significant reduction in these platelet activation in trichosanthes cucumerina was increasing the platelet count, RBC, lymphocytes and polymorphs are totally increased when compared the normal and cyclophosphamide groups.

6. CONCLUSION

Evaluation of the effect of increasing platelet count in Trichosanthes Cucumerina by using albino rats.d Ahmad, et al described the potential of snake guard fruit extract .The Platelets count (PLT), Red Blood Cells (RBC), lymphocytes, polymorphs and Leucocytes was Subsequently, increased in the blood sample was rechecked after the administration of fruit juice extract. From our Study on Evaluation of platelet augmentation activity of Snake gourd fruit juice extract in mice with cyclophosphamide induced thrombocytopenia showed significant increase in platelet count. It may be hypothesized that many active components of snake gourd extract, is the natural course of recovery with increasing the platelet count and prevents the complication of thrombocytopenia without any side.

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