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RESEARCH ARTICLE

POTENTIAL ROLE OF PUNICA GRANATUM JUICE FOR TREATING DENGUE FEVER ON CYCLOPHOSPHAMIDE INDUCED SWISS ALBINO MICE

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Abstract

The present work aims at analysing the potential of pomegranate juice which is used for the treatment of dengue fever as it is used in increasing the body's immune power by increasing the platelets count. The phytochemical characteristics of pomegranate juice extract is studied and compared with papaya leaf juice extract. Both the papaya leaves and pomegranate are naturally and easily available, which are non toxic, has no side effects. In our study, the effectiveness of pomegranate juice was studied by introducing the juice into cyclophosphamide induced swiss albino mice. Before the administration, the platelet count, White Blood Cells (WBC) and Red Blood Cells (RBC) was analysed, after the induction of cyclophosphamide platelet count and white blood cells decreased from 9,74,200, 11380 to 8,42,400, 6140 and no significance for RBC. The blood samples were rechecked after introduction of pomegranate juice extract. It was observed that platelet count, WBC increased significantly, but there was no significant increase in RBC. Therefore, it can be concluded that pomegranate juice significantly increases the platelet count and WBC.

Introduction:-

Dengue is the most deadly emerging viral diseases of human, and in recent decades it has become a major international public health concern. The dengue fever is one of the life threatening diseases caused by dengue virus that is borne and transmitted by mosquitoes living in tropical and subtropical climates worldwide, mostly in urban and semi-urban areas. Dengue infection produces a self limiting illness that is often characterized by sudden onset of fever, headache, fatigue, nausea, vomiting and rashes[1] . This disease also called as "break-bone" fever because it sometimes causes severe joint and muscle pain that feels like bones are breaking.

Dengue fever is found principally throughout and shortly after the rainy season in tropical and subtropical areas of the Central and South America, Africa, Southeast Asia and China, India, the Middle East, Australia and the South and Central Pacific. Dengue fever may be caused by one of the four types of dengue virus such as DEN-1, DEN-2, DEN-3, and DEN-4. The dengue virus (DENV) is the cause for dengue fever, it belongs to one of the five serotypes[2]. Dengue virus, mosquito-borne single positive-stranded RNAvirus, it belongs to the family Flaviviridae, genus Flavivirus[2,3]. It comes in the group IV ((+)ssRNA) with unassigned order , flaviviridea family , flavivirus

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genus. Dengue virus can be transmitted from the bite of an carrier mosquito, Aedes mosquito. Mosquitoes become infected when they bite infected humans, and can later transmit the infection to other people.

Two main species of mosquito, Aedes aegypti and Aedes albopictus, have been responsible for all cases of dengue. Dengue can be diagnosed by doing two blood tests, 2 to 3 weeks apart to indicate the presence of antibodies to the virus. However, in epidemics, a health care provider often diagnoses dengue "presumptively" by typical signs and symptoms without waiting for lab results.

One of the oldest known fruits, the pomegranate (Punica granatum) is an original native of Persia. Pomegranate contains range of chemicals that might have antioxidant effects. Some preliminary study indicated the presence of chemicals in pomegranate juice might slow the progression of hardening of the arteries and possibly fight cancer cells. Many studies show that the pomegranate is one of the most powerful, nutrient dense foods for overall good health. These clinical findings clearly show a correlation between pomegranate compounds and their positive effect on both human and animal cardiovascular, nervous, and skeletal health.

In our present study, pomegranate juice extract was considered so that it can be used as an alternative for the treatment of dengue disease. Lack of earlier reports to prove the benefits of pomegranate juice on the dengue juice offered sufficient scope to undertake this study. The ability of pomegranate juice extract in increasing the platelet count in the presence of inducer cyclophosphamide was evaluated on mice model.

Materials and Methods:-

Collection of sample:

Pomegranates were collected from APMC market, Tumkur, Karnataka state, India and it was stored in polyethylene bags.

Preparation of the juice:

Pomegranate peel was removed and seeds were collected .It was then weighed, juice was extracted using the mixer grinder. The extract was used for the further analysis.

Phytochemical Analysis:

Phytochemical tests were carried out qualitatively on both the juices specimens using standard procedures to identify the amino acids and phytochemical constituents[4].

Anti-Oxidant Analysis:

DPPH Radical Scavenging Assay:

DPPH radical scavenging assay is an excellent method of determination of antioxidant efficacy of compounds based on colorimetry[5] with some modifications. The reaction mixture consists, 1 ml of DPPH solution (0.1 mM), different concentrations of pomegranate juices ranging from 80µg - 400µg dissolved in 1 ml of methanol. The mixture was mixed properly and allowed for incubation in dark place for 30 minutes. The color change was observed and the absorbance was recorded spectrophotometrically at 520nm using methanol as blank solution. The extent of decreased absorbance indicates the antioxidant strength of a test sample. Standard ascorbic acid was used for reference and expressed in terms of its equivalents[6].

The strength of an antioxidant activity was calculated according to the formula,

$\textbf{\% of DPPH radical scavenging} = \frac{(\mathbf{A_{con}} - \mathbf{A_s})}{\mathbf{A_{con}}} \mathbf{X100}$ Where as, $\mathbf{A_{con}}$- Optical density of control and $\mathbf{A_s}$ - Optical density of test sample

Ferric Reducing Antioxidant Potential (FRAP):

The determination of the total antioxidant activity using FRAP assay in the extract followed after a modified method[4]. The stock solution included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$) at pH 3.6, 10 mM TPTZ (2,4,6-Tri Pyridyl-sTria Zine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in distilled water. Then acetate buffer (25 ml) and TPTZ (2.5ml) were mixed together with $FeCl_3 \cdot 6H_2O$ (2.5 ml). The temperature of the solution was raised to 37 ºC before it was used. Plant extracts (150 µl) were allowed to react with the FRAP solution (2.85 ml) for 30 min under dark conditions. The absorbance was measured at 593nm. The

standard curve was linear between 200 and 1,000μM $FeSO_4$. Results were expressed in μM Fe (II)/g dry mass and compared with the standard, ascorbic acid[7].

Anti-Inflammatory Test:
Membrane stabilization test:
Preparation of Red Blood cells (RBC's) suspension:
5ml of fresh whole human blood was collected and transferred to the centrifuged tubes containing EDTA to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed thrice with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Hypotonicity induced Human Red Blood Cell (HRBC) membranestabilization method:
The reaction mixture consists of 1.0 ml of test sample of different concentrations (40 μg – 200 μg) in 1ml of 0.2M phosphate buffer and 0.5 ml of 10% HRBC suspension, 0.5ml of 0.25 % hyposaline were incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac was used as standard and water was used as control instead of hyposaline to produce 100 % hemolysis without plant extracts. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following equation:
% of Hemolysis = (Optical density of test sample / Optical density of control) X 100
% Protection=100–[(Optical density of test sample/ Optical density of control) X100]

Heat induced Human Red Blood Cell (HRBC) membrane stabilization method:
The reaction mixture in heat induced hemolysis consists of 1.0 ml of test sample of different concentrations (40 μg – 200 μg) in normal saline and 1.0 ml of 10% RBC suspension. Diclofenac sodium was taken as a standard drug. Distilled water was used as control instead of normal saline to produce 100 % hemolysis without plant extracts. All the tubes containing reaction mixture were incubated in a water bath at 56^0 C for 30min. After incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following equation:
% of Hemolysis=(Optical density of test sample / Optical density of control) X 100
% Protection=100 –[(Optical density of test sample / Optical density of control)X100]

Albumin denaturation method:
Anti inflammatory activity involves inhibition of albumin denaturation. The reaction mixture consists of 1.0ml of distilled water and varying concentrations of the both juice samples and standard (80 μg – 400 μg), 0.2 ml of 0.05 % BSA and 1.8 ml of 0.2 M phosphate buffered saline solution (pH 6.4). The mixtures were incubated at 37ºC for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, the absorbance was measured spectrophotometrically at 660nm against a blank. Diclofenac sodium was used as standard drug and the percentage inhibition of protein denaturation was calculated by using the following formula[8]:

Relative % of inhibitory activity = $(A- A_{min}) / (A_{max} - A_{min}) \times 100$
Where, A – Abs of Sample ; A_{min} – Abs of control ; A_{max} – Highest Abs of Standard .The experiment was performed in triplicate

Animal studies:
Preparation of Punica granatum juice:
About 250 g of pomegranate fruit was taken and made into juice using mixer grinder. The juice was used for invivo studies on mice. Two doses of pomegranate juice sample (0.4 ml as low dose and 0.8 ml as high dose) were selected for administration.

For low dose, 36 ml of the pomegranate juice was used, 0.4 ml has given to each of five mice thrice in a day for six days of pre-treatment and for high dose 72 ml of sample was used, 0.8ml has given to each of five mice thrice in a day for three days of pre-treatment[9].

Twenty number of adult male Swiss albino mice (25-40g) were obtained from the animal house of Sree Siddaganga College of Pharmacy, Tumkur, India, which was maintained under 12:12 h light-dark cycle. These animals were randomized into four groups and housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They were given with standard pellet diet and water ad libitum throughout the experimental period. All animals were acclimatized to laboratory conditions for atleast a week before commencement of the experiment. The experiments were conducted according to the agreed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and were approved by the Institutional Animal Ethical Committee (IAEC) of Sree Siddaganga College of Pharmacy, Tumkur, Karnataka (**SSCP/IAEC. Clear/142/2013-14**).

Evaluation of Punica granatum in Cyclophosphamide induced sickness behavior:
The male mice were selected and randomized into four groups containing five animals each (n=5). The animals were pre-treated for 3days before being challenged with Cyclophosphamide.

1. **Group 1 (Saline):** Group 1 was treated with Saline (10 ml/kg). This group served as normal control.
2. **Group 2 (Cyclophosphamide):** Group 2 was treated with Cyclophosphamide (50 mg/kg I.P.) on 2nd day. This group served as disease control.
3. **Group 3 (Pomegranate + Cyclophosphamide):** Group 3 was treated with Punica granatum (0.4 ml) and challenged with Cyclophosphamide (50mg/kg I.P.) on 2nd day. This group served as low dose control.
4. **Group 4 (Pomegranate + Cyclophosphamide):** Group 4 was treated with Punica granatum (0.8 ml) and challenged with Cyclophosphamide (50mg/kg I.P.) on 2nd day. This group served as high dose control.

Table 1:- Grouping of the animals>.

Groups	Treatment
I (Normal control)	Saline (10ml/kg)
II (Disease control)	Cyclophosphamide (50 mg/kg i.p.)
III (Disease + Drug)	Pomegranate (0.4 ml/mice/day) + Cyclophosphamide (50mg/kg i.p.)
IV (Disease + Drug)	Pomegranate (0.8 ml/mice/day) + Cyclophosphamide (50mg/kg i.p.)

Figure 1:- Grouping of the mice.

The experiment was conducted for seven days. Grouping of mice followed by randomization was done on first day. On second day of study, treatment was based on disease control, high dose, and low dose. In subsequent days, mice blood sample was collected by using Retro-Orbital method under anesthesia (diethyl ether) followed by estimation of platelet count, WBC and RBC.

Body weights of all the mice were recorded before starting the treatment and at the end of the treatments. The platelet count, WBC and RBC was measured on alternate days between 9 to 11 A.M using cell counter (Lablife H3D premier automated hematology analyzer) available in the laboratory of Shridevi Institute of Medical Sciences and Research Centre, Tumakuru, Karnataka.

Results:-

Phytochemical analysis was checked for the pomegranate juice extracts. The results of phytochemical analysis are shown in the table 2.

Table 2:- Phytochemical analysis.

Test	Pomegranate Juice
Alkaloids	–
Flavonoids	–
Tannin	+
Saponin	+
Cardiac glycosides	+

(Note: [+] indicates presence, [-] indicates absence)

Anti-oxidant analysis:

The amount of antioxidant present in pomegranate juice is shown in the following graphs.

DPPH Radical Scavenging Assay:

DPPH assay was carried out on pomegranate juice extract. Ascorbic was taken as standard. Results of the DPPH assay is shown in the below table 3.

Table 3:- DPPH Radical Scavenging Assay.

Concentration(µl)	Ascorbic acid (%)	Pomegranate juice (%)
50	80.16	19.91
100	80.97	21.02
150	81.78	23.43
200	81.99	30.05
250	82.02	34.85
300	82.55	39.86

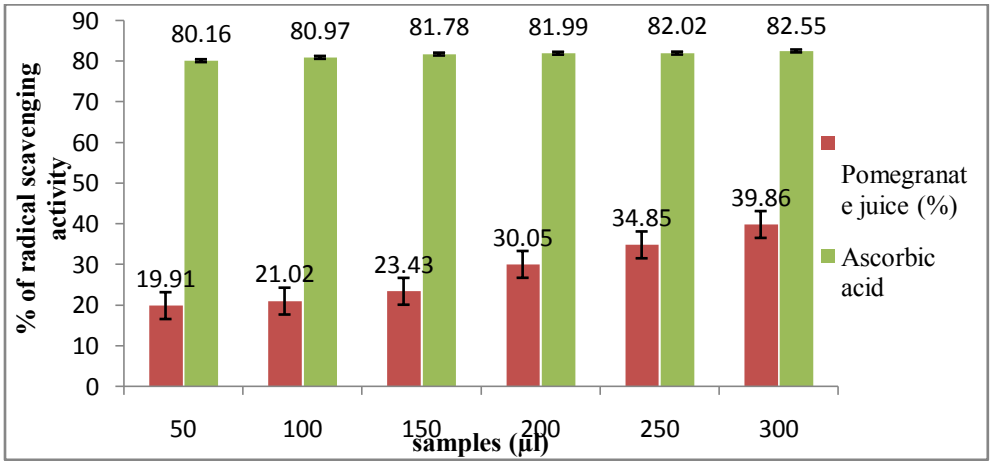

Figure 2:- DPPH Radical Scavenging Assay.

Ferric Reducing Antioxidant Potential (FRAP):

FRAP was carried out on pomegranate juice extracts. Ascorbic was taken as standard. Results of the FRAP is shown in the below table 4.

Table 4:- Table showing the results of FRAP analysis**.**

Concentration (µl)	Ascorbic acid	Pomegranate juice
40	724	96
80	620	230
120	1068	399

160	1356	462
200	1688	659

Anti- inflammatory analysis:

Hypo tonicity induced HRBC membrane stabilization method:

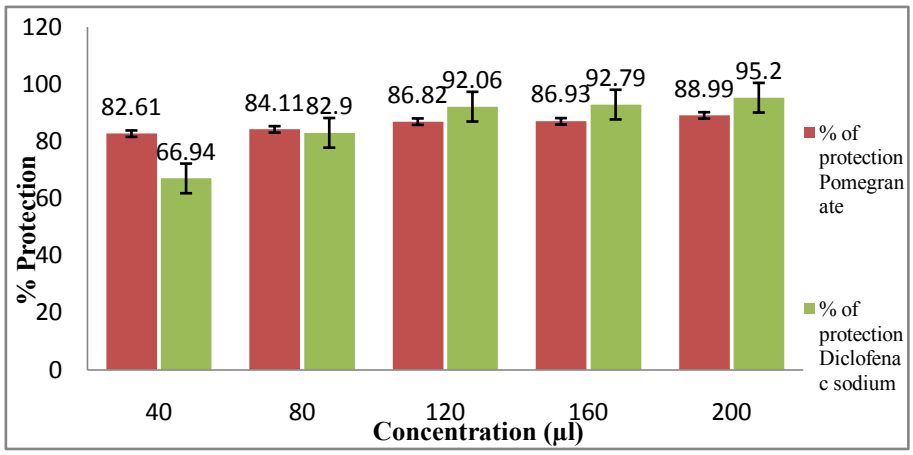

Figure 3:- Hypotonicity induced HRBC membrane stabilization method.

Heat induced HRBC membrane stabilization method:

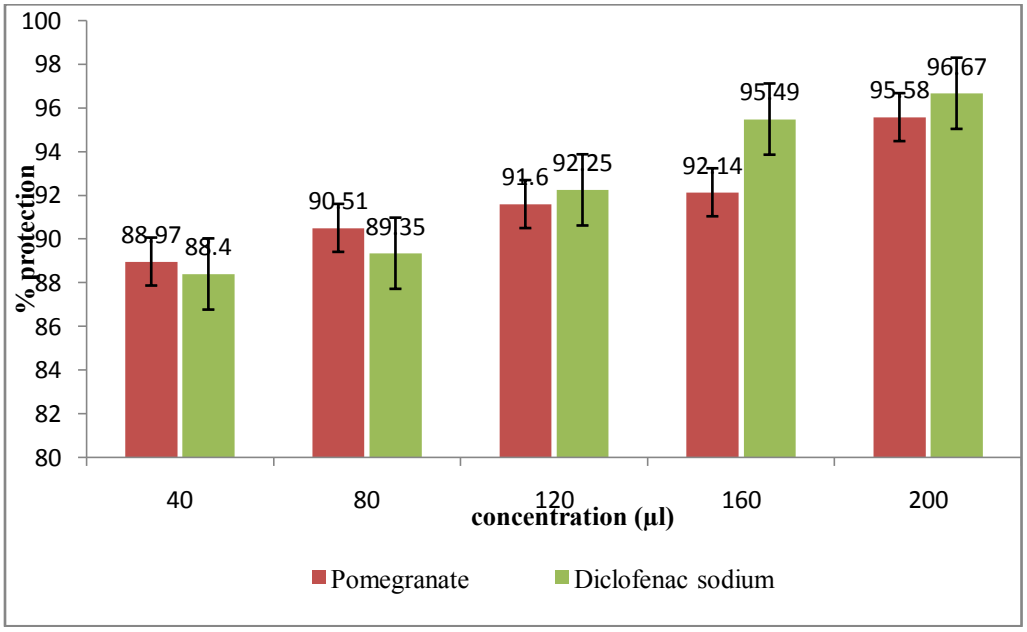

Figure 4:- Heat induced HRBC membrane stabilization.

Albumin denaturation method:

Albumin denaturation method was carried out on pomegranate juice. Ascorbic was taken as standard. It has shown that denaturation of the albumin is less in pomegranate juice. Results of the albumin denaturation is shown in the below table 5.

Table 5:- Albumin denaturation method.

Concentration (µl)	Standard (%)	Pomegranate juice (%)
80	78.24	69.28
160	80.29	71.34
240	84.66	73.64

320	86.84	75.92
400	89.37	77.39

Mice studies:

Platelet Count:

Table 6:- Platelet count for normal, disease, low dose and high dose control.

Normal Control (up to six days)				
Mice number	Normal saline in ml (daily)	Day 2	Day 4	**Day 6**
1	0.8	1182X10^3	1239X10^3	**1298X10^3**
2	0.8	825 X10^3	1135 X10^3	**1230 X10^3**
3	0.8	716 X10^3	810 X10^3	**1010 X10^3**
4	0.8	993 X10^3	1274 X10^3	**1543 X10^3**
5	0.8	905 X10^3	1113 X10^3	**1226 X10^3**

Disease Control (up to six days)				
Mice number	Cyclophosphamide mg/kg (only once)	Day 2	Day 4	**Day 6**
1	50	1160X10^3	954 X10^3	**889 X10^3**
2	50	887X10^3	784 X10^3	**723 X10^3**
3	50	962 X10^3	947 X10^3	**943 X10^3**
4	50	964 X10^3	932 X10^3	**899 X10^3**
5	50	898 X10^3	876 X10^3	**758 X10^3**

High Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the Cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.4	960 X10^3	958 X10^3	**1344 X10^3**
2	0.4	1197 X10^3	1119 X10^3	**1914 X10^3**
3	0.4	1037 X10^3	998 X10^3	**1193 X10^3**
4	0.4	1415 X10^3	1335 X10^3	**1192 X10^3**
5	0.4	1047 X10^3	984 X10^3	**1406 X10^3**

Low Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the Cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.8	754X10^3	612 X10^3	**1279X10^3**
2	0.8	641X10^3	574 X10^3	**1461 X10^3**
3	0.8	804 X10^3	669 X10^3	**1285 X10^3**
4	0.8	812 X10^3	668 X10^3	**1549 X10^3**
5	0.8	833 X10^3	621 X10^3	**1268 X10^3**

(Note: Standard form of Cyclophosphamide (50mg/kg) has given only once to each mice)

WBC Count:

Table 7:- WBC count for normal, disease, low dose and high dose control.

Normal Control (up to six days)				
Mice number	Normal saline in ml (daily)	Day 2	Day4	**Day 6**
1	0.8	5.9 X10^3	5.6 X10^3	**10.7 X10^3**
2	0.8	6.7 X10^3	6.6 X10^3	**6.7 X10^3**
3	0.8	9.3 X10^3	10.2 X10^3	**8.6 X10^3**

4	0.8	8.6 $X10^3$	8.3 $X10^3$	**9.0 $X10^3$**
5	0.8	6.7 $X10^3$	7.4 $X10^3$	**7.0 $X10^3$**

Disease Control (up to six days)				
Mice number	Cyclophosphamide mg/kg (only once)	Day 2	Day4	**Day 6**
1	50	10.4$X10^3$	9.0 $X10^3$	**6.5 $X10^3$**
2	50	10.4 $X10^3$	5.6 $X10^3$	**5.3 $X10^3$**
3	50	10.6 $X10^3$	7.6 $X10^3$	**6.8 $X10^3$**
4	50	11.7 $X10^3$	10.9 $X10^3$	**6.0 $X10^3$**
5	50	13.8 $X10^3$	8.8 $X10^3$	**6.1 $X10^3$**

High Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the Cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.8	4.2$X10^3$	9.1 $X10^3$	**8.5 $X10^3$**
2	0.8	2.9 $X10^3$	6.4 $X10^3$	**12.0 $X10^3$**
3	0.8	4.5 $X10^3$	8.30 $X10^3$	**7.1 $X10^3$**
4	0.8	15.8 $X10^3$	14.6 $X10^3$	**13.4 $X10^3$**
5	0.8	5.0 $X10^3$	9.7 $X10^3$	**8.3 $X10^3$**

Low Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the Cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.4	3.9$X10^3$	7.3$X10^3$	**5.8$X10^3$**
2	0.4	3.3$X10^3$	6.3$X10^3$	**6.0$X10^3$**
3	0.4	6.7 $X10^3$	5.4$X10^3$	**6.4$X10^3$**
4	0.4	6.7 $X10^3$	5.4$X10^3$	**6.4$X10^3$**
5	0.4	5.7 $X10^3$	6.1$X10^3$	**5.5$X10^3$**

(Note: Standard form of Cyclophosphamide (50mg/kg) has given only once to each mice)

RBC Count:

Table 8:- RBC count for normal, disease, low dose and high dose control.

Normal Control (up to six days)				
Mice number	Normal saline in ml (daily)	Day 2	Day4	**Day 6**
1	0.8	8.67 $X10^6$	8.90 $X10^6$	**9.09 $X10^6$**
2	0.8	7.16 $X10^6$	8.31 $X10^6$	**9.45 $X10^6$**
3	0.8	9.96 $X10^6$	8.22 $X10^6$	**9.28 $X10^6$**
4	0.8	9.23 $X10^6$	8.26 $X10^6$	**6.12 $X10^6$**
5	0.8	8.80 $X10^6$	7.00 $X10^6$	**7.85 $X10^6$**

Disease Control (up to six days)				
Mice number	Cyclophosphamide mg/kg (only once)	Day 2	Day4	**Day 6**
1	50	7.72 $X10^6$	7.99 $X10^6$	**9.80 $X10^6$**
2	50	8.11 $X10^6$	9.93 $X10^6$	**7.64 $X10^6$**
3	50	8.34 $X10^6$	8.30 $X10^6$	**9.46 $X10^6$**
4	50	8.13 $X10^6$	8.39 $X10^6$	**8.30 $X10^6$**
5	50	8.69 $X10^6$	7.50 $X10^6$	**7.76 $X10^6$**

Low Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.4	9.05 $X10^6$	8.34 $X10^6$	**7.51 $X10^6$**
2	0.4	8.28 $X10^6$	8.02 $X10^6$	**7.17 $X10^6$**
3	0.4	9.20 $X10^6$	9.23 $X10^6$	**7.06 $X10^6$**
4	0.4	7.62 $X10^6$	9.10 $X10^6$	**6.82 $X10^6$**
5	0.4	9.97 $X10^6$	7.86 $X10^6$	**7.40 $X10^6$**

High Dose (up to six days)				
Mice number	Pomegranate Sample Dosage in ml (daily)	Normal platelet Count /µl	Platelet count after inducing the cyclophosphamide /µl	**Platelet count after inducing the pomegranate sample /µl**
1	0.8	9.31$X10^6$	6.94 $X10^6$	**8.22 $X10^6$**
2	0.8	9.63 $X10^6$	7.10 $X10^6$	**6.18 $X10^6$**
3	0.8	9.14 $X10^6$	8.87 $X10^6$	**7.49 $X10^6$**
4	0.8	8.98 $X10^6$	8.87 $X10^6$	**7.44 $X10^6$**
5	0.8	9.78 $X10^6$	8.28 $X10^6$	**6.58 $X10^6$**

(Note: Standard form of Cyclophosphamide (50mg/kg) has given only once to each mice)

Dengue has the effect on 40 % of the population, many attempt have been made to identify the suitable drug for the treatment of dengue. Our study indicated that pomegranate juice has the potential to increase the platelet count, as the juice had the potential medicinal benefits.

In the present study, we used fresh **pomegranate juice extracts** without adding any chemical. Phytochemical analyses have indicated that pomegranate juice extract contains chemical compounds such as tannins, saponins, and cardiac glycosides. These compounds can affect various biological processes in the body in ways that might have harmful or beneficial effects.

Antioxidant assays such as DPPH radical scavenging assay and FRAP method was carried out using ascorbic acid as the standard.

Also test was carried out to evaluate the in-vitro anti-inflammatory activity of the pomegrante juice samples. The human Red Blood Cell membrane is analogous to the lysosomal membrane [21] and the stabilization of the RBC indicates the stabilization of lysosomal membranes. Stabilization of these RBC and lysosomal cells inhibits lysis and it releases the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response. Exposure of Red Blood Cells to hypotonic medium, heat, injurious substances such as methyl salicylate or phenylhydrazine results in the lysis of membranes accompanied by haemolysis and oxidation of haemoglobin [10,11]. The concentrations of the percentage of membrane stabilization by Hypotonicity induced were more effective in pomegranate juice (86%) with Diclofenac Sodium as a reference standard (87%). The percentage of membrane stabilization by Heat induced membrane stabilization was found to be 92% for pomegranate juice and 93% for the reference Diclofenac Sodium.

The pomegranate juice has a similar role to that of papaya leaf juice extract in the increase of platelet count. However, the benefits in the dengue have not been tested technically. Therefore, it is essential to check the benefits of the juices on the animal model. Pomegranate juice sample was stored at -19^{0}C.Animal studies have been conducted on adult male Swiss Albino mice (25-40 g) of 20 in numbers using liquid form of the juice. This study clearly showed increasing platelet and WBC in healthy mice after injecting the pomegranate juice. These animals remained healthy with normal weight gain during the experiment.

All mice (both control and test) survived until the scarification. Oral feeding of the juice with 0.4ml as low dose/mouse/3 times a day for 6 days and 0.8ml as high dose/mouse/3 times a day for 6 days had no adverse effects

on animal behavior, appetite and bodyweight. By feeding at different doses for a long period of time , the action of juice extract was studied. Study indicated that there was a significant rise in some haematalogical parameters, such as platelet and WBC counts, only in the test group. Other parameters, such as red blood cell count did not show significant increase in either group.

Initially, platelet counts in the test and control groups were 924.2 $\times 10^3/\mu L$ and 974.2 $\times 10^3/\mu l$ respectively and $1368.4 \times 10^3/\mu l$ and $1409.8 \times 10^3/\mu l$ respectively at the end of the experiment. The effect on platelet counts by oral administration of **pomegranate juice** extract is statistically significant. In addition, the WBC count in the test group increased significantly in comparison with the control.

Therefore, it is clear that an oral feeding of juice extract causes considerable increases in platelet and WBC counts in the animal model without causing any acute/subacute toxicity. Therefore, **pomegranate juice** extract may be used as a medicine to boost haemopoiesis and thrombopoiesis when these have been suppressed by dengue disease. However, this is a preliminary study and more work is needed to isolate and to identify the biologically active ingredients of **pomegranate juice** that are responsible for these effects.

Conclusion:-

From our study, it is clear that fresh P.granatum juice extract significantly increased the platelet and WBC counts in the test group as compared to controls; there is no significant increase in the RBC count. The study was made on animal model which statistically indicated positive effect without any acute/ sub acute toxicity. Therefore, from our study made on swiss albino mice with cyclophosphamide induction, it is concluded that the pomegranate juice can be used as a medication to increase the platelet count and hence to cure the dengue fever.

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