EVALUALTION OF CARICA PAPAYA LEAF EXTRACT ON MEGAKARYOCYTES COUNT: AN ANIMAL MODEL

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ABSTRACT

Objective: To determine the effect of Carica Papaya leaf extract on megakaryocytes count in animal model.

Material and Methodology: Laboratory experimental study was designed to achieve the objective and conducted between July and December 2021. Sample size was calculated by using G power with an effect size of 0 .25 and alpha 0.05. The total sample size was 25 which were divided into five groups. Methanolic extract of Carica Papaya leaves was prepared. Thrombocytopenia was induced and extract was given in doses of 250 and 500 mg/Kg. The blood was taken, and slides were prepared. The mean megakaryocytes count was measured per visual field of microscope. Mean values were calculated using Microsoft Excel 2016 and compared within the groups using paired T-test through Statical Package for Social Sciences (SPSS) version 21.

Results: A statistically significant increase in megakaryocytes count was observed with increasing dose of Carica Papaya leaf extract as compared to the control groups (*p*-valvue < 0.05).

Conclusion: Our study concluded that Carica Papaya extract significantly increases the number of megakaryocytes count in animal model. However, further large-scale studies are required to evaluate its effects on human body.

Keywords: Carica Papaya, thrombocytopenia, megakaryocytes, dengue.

Introduction

Megakaryocytes (MKs) are the largest cells in the bone marrow, with an average size of 50 – 100 µm, but they account for less than 0.1% of nucleated cells. Pluripotent HSCs are responsible for the production of megakaryocytes (MKs), which roughly produces1x10^{11} platelets per day, that can be increase 10-20 folds during times of high demand and when thrombopoietin-mimetic medications are used to stimulate platelet production. Thrombocytopenia is a decrease in platelet count below 150,000 platelets/µL, a condition that raises the risk of potentially fatal internal bleeding (1). The causes of thrombocytopenia may be classified into congenital and acquired types.

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It can also be caused by various acquired abnormalities such as a decrease in megakaryocytic precursors, ineffective thrombopoiesis, or abnormal thrombopoiesis regulation (2).

Carica is a genus of a tree-like herbaceous plant that belongs to the Caricaceae family, which is a small family of flowering plants. The papaya plant originated in the eastern Central America, but now grown in all tropical and sub-tropical regions around the world. The plant is evergreen, with male and female flowers produced on separate plants. Papaya fruits have a high nutritional value because they are high in provitamin-A and ascorbic acid (3). Plant medicinal activities are attributed to active chemical compounds. Tannin, saponin, alkaloids, flavonoids, and glycosides are found in papaya leaves, while minerals such as Ca, Fe, Mg, K, Zn, and Mn are found in the shoots (4). C. papaya has a variety of pharmacological activities such as antioxidant, insecticidal, antimicrobial, antidiarrheal, wound healing, anti-neoplastic, anti-malarial and thrombopoietic actions (5).

Diseases such as dengue cause a low thrombocyte count in the blood, require a rapid response in terms of thrombocyte levels. The extract of papaya leaves, which is high in papain, has been proven to boost thrombocyte count in dengue patients as well as in a murine animal model. The phenolic acids, carpaine and flavonoids are found to be responsible for thrombopoietic effect (6). The juice of C.Papaya fruit increase the release of thrombopoietin

(TPO), through increase synthesis of interleukin-6 (IL-6), which induces the production of thrombocytes (7). Furthermore, C. Papaya leaf extract causes a significant increase in platelets count in patients receiving cancer chemotherapy (8). Since the production of thrombocytes significantly depends upon the megakaryopoiesis, therefore, the aim of our study was to evaluate the effect of Carica papaya leaf extract on megakaryocyte count in drug-induced thrombocytopenia in animal models.

Material and Methods

A laboratory experimental study was conducted in the Animal house and pharmacology laboratory while the investigations were carried out in the Histopathology Laboratory of Peshawar Medical College after obtaining the ethical approval by the ethical committee of Peshawar Medical College, Peshawar. This six-month study was conducted from 1st July 2021 till 31 December 2021. A simple random sampling technique was used. Sample size was calculated by using G-power with an effect size of 0 .25, alpha 0.05 and power of the study as 95%. Total sample size calculated was 25 i.e., a total five groups were made with 5 mice in each group (**Table 1)**. Swiss albino mice were used. Inclusion criteria was young adult mice of either sex age between 8–12-week and weight between 23-35 grams while pregnant or sick mice were excluded from the study. Animals were procured from the Animal house at Veterinary Research institute (VRI) Peshawar and were housed in polypropylene cages. The laboratory conditions were kept standardized with a relative humidity of 55-60%, temperature 25±2 °C and a 12-hour light and 12 hours dark cycle. Mice were fed with pellets diet and water ad libitum. Cyclophosphamide injection (Cyclomide® 1gm) was purchased from local market and was dissolved in 50 ml of distilled water to induce thrombocytopenia. Hydrocortisone (Solu-cortef®) injection (1g) was obtained from local market. Insulin syringes were used for giving dosages of extract. Analytical Methanol 99.9% and distilled water were obtained from Sigma®.

Fresh green mature leaves of C. papaya were collected from a local nursery in Hayatabad, Peshawar. After shade drying, the leaves were grinded into fine powder and stored in dried bottles. The methanolic extract of the powdered plant material was prepared by soaking the fine powder of C. Papaya weighing 1kg into 2L of methanol (analytical 99.9%) with a ratio of 20:80 for 10 days. The solution was continuously stirred. The solution was then filtered through Whatman filter paper No: 1 which was further concentrated using a rotary evaporator, maintained at 55°C, at Pakistan Council of Scientific and Industrial Research (PCSIR) laboratory, Peshawar. The resulting C. Papaya extract was a dark green powder. Approximately 200g of C. Papaya extract was obtained from 1Kg of dried leaves powder and was stored in amber coloured bottles at 4°C for further use. Two different solutions of C. Papaya extract were then prepared for use in the current experimental study i.e., 250 mg/ml and 500 mg/ml by dissolving in distilled water. A 50 mg/kg body weight of cyclophosphamide dissolved in normal saline was injected to each mouse for the induction of thrombocytopenia. The drug was given once every day for 3 consecutive days. Thrombocytopenia was confirmed by taking blood from tail of mice and estimation of bleeding time (9). Doses of the leaf extract were given to all the mice and kept under observation (10). For histological analysis, the mice were sacrificed, and their femur bones were taken out. They were kept in 10% formalin to preserve the cells. After that they were placed in 3% Nitric oxide for 3 to 5 hours for decalcification. Once decalcification was accomplished the bone were reduce in sections with the assist of surgical blade number 24.The histologic arrangements were made in Histopathology Laboratory, Peshawar Medical College, Peshawar. The histological arrangements were made on haematoxylin-eosin. The H& E-stained slides were then studied beneath the microscope at 100X magnification and megakaryocytes were count. Each slide was seen in 3 visual fields (11). The results were shown as the number megakaryocytes per visual field. Data was entered and analysed in the Statistical Package for Social Sciences (SPSS v21). Quantitative variables presented as mean and standard deviation whereas categorical variables computed as frequencies and percentages. Paired sample T test was used to measure the difference between and within groups. A *p*-value equal or less than 0.05 was considered statistically significant.

Table 1: Division of Groups (n= 25)

Group No.	Group Name	Group Description
I.	Normal Control	Normal Saline given orally
II.	Negative Control	Cyclophosphamide 50mg/kg BW (Intra peritoneally)
III.	Positive Control	Cyclophosphamide 50mg/kg BW+ 0.5mg/kg BW Hydrocortisone
IV.	Experimental Group 1	Cyclophosphamide 50mg/kg BW was given for three consecutive days + Plant extract 250mg/kg BW was given on 7th day onwards till 14th day
V.	Experimental Group 2	Cyclophosphamide 50mg/kg BW was given for three consecutive days +Plant extract 500mg/kg BW was given on 7th day onwards till 14th day

RESULTS

All experiments were conducted on normal healthy mice. The Data obtained from H & E-stained slides were visualized under the microscope under three different fields showing difference in mean megakaryocyte count. As per our results, there was a decrease in mean megakaryocyte count in negative control group as compared to normal control group (9.3 vs. 3.36). On the other hand, the experimental groups (IV & V) receiving cyclophosphamide (50mg/kg) initially and *C.Papaya* leaf extract later in doses of 250mg/kg and 500mg/kg showed a gradual increase in their mean megakaryocyte count. The results are shown in **Table 2**. According to our results, an increase in megakaryocytes count in experimental groups i.e., IV and V , was observed as compared to the control groups. As per our results, it was found that there was a significant difference in mean megakaryocytes count between the experimental groups IV and the normal control group (***p*-value < 0.05**). However, a non-significant difference was observed in mean megakaryocytes count between the group V and normal control group. The results are shown in **Table 3**. The mean values of increase in megakaryocytes count in experimental groups (IV and V) and of negative control group. According to our results, the megakaryocytes count was found to be rising as the dose of C.Papaya extract was increasing as compared to negative control group samples. As per our results, there was a significant difference between the increase in megakaryocytes count in experimental groups V and the negative control group (***p*-value <0.05**), while no significant difference was found between the group IV experimental group and negative control group (***p*-value >0.05**) (**Table 4).** Finally, the mean values of increase in megakaryocytes count in experimental groups and of positive control were also compared. As per our results, there was a significant difference in megakaryocytes count between the experimental groups and the positive control group (***p*-value <0.05**), while no significant difference was found between the experimental group IV and positive control group (***p*-value >0.05**). According to our results, there was a gradual increase in megakaryocytes count as the dose of *C.Papaya* extract was increased within the experimental groups. The results are shown in **Table 5**.

Table 2: Descriptive Analysis of Groups (n=35)

Megakaryocyte count	Group 1 Normal Saline	Group 2 Cyclo	Group 3 Cyclo + Hydrocortisone	Group 4 Cyclo + 250mg C.P	Group 5 Cyclo + 500mg C.P
1.	9.3	3.3	5.3	5.3	11.8
2.	9.3	3.3	4.6	4.3	12
3.	10	3.3	4.3	5	9.9
4.	9.3	3.3	4.6	7	10.1
5.	8.6	3.6	4.3	5.6	10.7
Mean	9.3	3.36	4.62	5.44	10.9

Table 3: Comparison of Megakaryocyte Count between Normal Control Group & Experimental Groups

		Mean	Std. Deviation	t-value	Sig. (2-tailed)
Pair 1	Group4 (Cyclo+250mg)	5.44	1.00	-7.17	**0.02**
	Group1 (Normal control)	9.30	0.49		
Pair 2	Group5 (Cyclo+500mg)	10.90	0.96	2.97	0.41
	Group1 (Normal control)	9.30	0.49		

Table 4: Comparison of Megakaryocyte Count between Negative Control Group & Experimental Groups

		Mean	Std. Deviation	t-value	Sig. (2-tailed)
Pair 1	Group4 (Cyclo+250mg)	5.44	0.996	4.68	0.09
	Group2 (Negative control)	3.36	0.134		
Pair 2	Group5 (Cyclo+500mg)	10.90	0.962	17.09	**0.000**
	Group2 (Negative control)	3.36	0.134		

Table 5: Comparison of Megakaryocyte Count between Positive Control Group & Experimental Groups

		Mean	Std. Deviation	t-value	Sig. (2-tailed)
Pair 1	Group4 (Cyclo+250mg)	5.44	1.00	1.70	0.165
	Group3 (Positive control)	4.62	0.41		
Pair 2	Group5 (Cyclo+500mg)	10.90	0.96	18.17	**0.000**
	Group3 (Positive control)	4.62	0.41		

DISCUSSION

Thrombocytopenia is a common haematological abnormality seen in patients with either mild or severe dengue virus (DENV) infection (12). Patients may require platelet transfusions to maintain normal haemostatic activity (13). Pakistan is a temperate and sub-tropical country that is a centre for numerous vector-borne diseases, including dengue fever and dengue haemorrhagic fever (14). In Pakistan, four dengue serotypes are present and circulate throughout the year, with the greatest outbreak occurring between September and November. The first dengue fever case was recorded in the late 1980s, indicating that the virus was prevalent in Pakistan (15). As of 2016, 71649 cases had been reported, with 797 deaths (16). There is currently no effective treatment for thrombocytopenia. However, platelet transfusions are currently the most efficient strategy to minimize intense hazards of profound thrombocytopenia (17). Since there are several complications concerning platelet transfusion therapy, there is a need for alternative ways to decrease the demand for this blood component.

C.Papaya fruit is used as a food and good source of nutrients. It also increases the production of thrombocytes from the bone marrow. According to a Malaysian study conducted by Subenthiren et al., when C.Papaya juice was given to patients suffering from dengue fever, a rapid and significant increase in platelets count was found after 40-48hrs of administration of the extract to the patients (18). In current study, the mean increase in megakaryocytes count was measured in three different visual fields of microscope. It was observed that when C.Papaya extract was administered in increasing doses to different experimental groups, a gradual and constant increase in megakaryocytes count was observed at different doses i.e., 250mg/Kg and 500mg/Kg. A maximum mean increase in number of megakaryocytes count was measured when at a dose of 500 mg/Kg i.e., 10.90 per visual field of microscope. Previously, Jorum et al., also demonstrated that the increasing doses of C.Papaya leaf extract causes rapid increase in platelets count in rats (19).

When these experimental group, were statistically compared with normal control group using paired sample T-test, a significant difference in megakaryocytes count was

observed (p-value ≤ 0.05). Furthermore, these experimental groups were also statistically compared with negative control group, the increase in megakaryocytes count was significantly different in experimental groups than the negative control group (p-value < 0.05). similarly, when the experimental groups were compared with positive control group, these experimental groups were found statistically different from the positive control group (p-value < 0.05).

The exact mechanism by which the C.Papaya leaves extract increases the platelet count is unknown. However, Aziz et al., found that when the extract was administered to peripheral blood leukocytes, it dramatically boosted interleukin-6 secretion. These researchers anticipated that IL-6 could raise platelet count both directly and indirectly by increasing the production of hematopoietic progenitors and thrombopoietin synthesis in liver cells (7). According to Afzan et al., there are four types of flavonoids identified in C. Papaya leaves extract i.e., quercetin, kaempferol, quercetin 3-rutinoside, and kaempferol 3-rutinoside (20). In mice, quercetin administration increased mRNA expression of stem cells factor (SCF) and erythropoietin (Epo) in bone marrow. Quercetin is known to boost the production of HSCs, promoting the differentiation of HSCs into megakaryocytes (21).

Conclusion

C.Papaya extract has a potential effect on increasing the number of megakaryocytes and platelets count. The potential use and safety of it is yet to be explored in humans. Therefore, large scale studies are required to elucidate the safe and effective doses of C.Papaya extract in humans.

Authors Contribution

Shahida Batool (SB): Manuscript writing
Shafaq zafar (SZ): Sample collection and manuscript writing
Sadat Aalam (SA): Sample collection and laboratory work
Shahid Fareed (SF): Data analysis and manuscript compilation.
Ayesha Iftikhar (AI): Results compilation and statistical analysis
Mohsin Ali (MA): Laboratory work and manuscript writing

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Conflict of Interest

The authors have no potential conflict of interest relevant to this article to report.

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