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ANTIPLATELET AND ANTIHEMOLYTIC ACTIVITIES OF HYDROETHANOLIC EXTRACT OF *ANNONA MURICATA* LEAVES

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ABSTRACT: This study designed to evaluate the antiplatelet and antihemolytic activity of hydroethanolic extract of *Annona muricata* leaves (HEEAM). The platelet aggregation assay was carried out using platelet-rich plasma, and the antihemolytic assay was done by hydrogen peroxide with different concentration of plant extract (100-500 µg/ml). HEEAM was found to possess more hydrogen donating ability when compared with the standard drug (aspirin). In addition, extract showed significant antihemolytic action (IC_{50} = 64.94 ± 0.01 µg/ml) against hydrogen peroxide (H_2O_2) induced hemolysis. It measures the degree of hemolysis when generated free radical damage. These were evident that the extracts exhibited strong antioxidant activity by protecting the red blood cells from hemolysis. Furthermore, the antiplatelet activity of HEEAM showed a maximum at 500 µg/ml concentration. It represents a promising treatment for thrombotic diseases. The percentage of hemolysis and platelet aggregation was calculated with standard aspirin. In conclusion, these observations suggest that HEEAM to posses a significant antiplatelet and antihemolytic potential.

INTRODUCTION: Natural medicines and their related research have created an interest in the pharmaceutical world. It has been recognized that natural products make them favorable as lead structures for drug discovery and differentiates from the collection of synthetic and combinatorial chemical compounds. Modern medicinal methods in genomics and structural biology provide a clear structure of diverse proteins from naturally derived molecules. Natural products will undergo continual urgent need to develop effective drugs and also play a primary role in the discovery of a new drug for treating various diseases [1].

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Platelets are active in homeostasis and thrombosis mechanism. Platelet also detects a disruption in the lining of a blood vessel and has a self-protecting mechanism that involves the formation of blood clots. Apart from its role in hemostasis, platelets also play a role in inflammation, antimicrobial activity, angiogenesis, tumor growth, and metastasis. Platelets are able to perform these functions in the presence of their specific receptors [2]. Several antiplatelet drugs have been recognized to inhibit platelet activity in acute thrombotic situations as well as to prevent adverse events.

Antiplatelet therapy is one of the most effective therapies for the treatment of atherothrombotic disease. Recently several studies have demonstrated the role of platelets in atherosclerosis and long-term use of antiplatelet drugs could also be postulated to slow down the progression of the disease [3]. Erythrocytes destruction induces hemolysis by which hemoglobin and other internal

cell components are released into the surrounding fluids. Due to the majority of polyunsaturated fatty acids in erythrocyte membranes, they are highly susceptible to oxidative damage, whose consequence is hemolytic process [4]. *Annona muricata* L., commonly known as graviola or soursop, belongs to the family Annonaceae. Traditionally, the leaves were used in the treatment of headaches, insomnia, cystitis, liver problems, diabetes, and hypertension also proved to possess various beneficial effects [5]. The aim of this study was to investigate the antiplatelet activity and antihemolytic activity of *Annona muricata* leaf extract and to identify promising alternatives to treat various diseases associated with haemolysis.

MATERIALS AND METHODS:

Collection and Extraction of Plant Material: The leaves of *Annona muricata* were collected from Coimbatore. The leaves were authenticated by the Head, Botanical Survey of India, in Tamil Nadu Agricultural University. Coimbatore (BSI/SRC/5/ 23/2015/Tech/153). The leaves were washed with running water to remove dust and sand and shade dried at room temperature. The dried leaves were grounded into fine powder kept in an airtight container for further analysis.

Preparation of Plant Extract: 25 g of the leaf powder was weighed and soaked separately in 250 ml of hydro ethanol, in the ratio of 1:10 weight by volume (w/v). These were allowed to agitate manually and left undisturbed for 72 h. It was then filtered using Whatman No.1 Filter paper, and filtrates were evaporated. The extracts were stored at 4 °C until further processing.

Antiplatelet Activity: Platelet-rich plasma and tyrode buffer were used for the antiplatelet activity, according to Iman *et al.* 2006 [6]. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 22 °C for 6 min; at 400 g. Platelets were adjusted to 3.0×10^8 cell/ml with sterile saline. Prepared Tyrode buffer by using sodium chloride (149 mM), potassium chloride (2.6 mM), sodium bicarbonate (9.5 mM), glucose (5.5 mM), sodium dihydrogen phosphate (0.5 mM), magnesium chloride (0.6 mM) and gelatin(0.25%). The platelet-rich plasma 0.13×10^{-7} for each assay was resuspended in Tyrode buffer (pH adjusted to 7.4 with 0.25 M HCl).

Aggregation of the platelets was induced by using $CaCl_2$ at a final concentration of 2 μM. Platelet aggregation was recorded by increasing the transmittance value of spectrophotometric measurements at 415 nm. To determine the *in-vitro* antiplatelet aggregation property, different concentrations (100, 200, 300, 400 and 500 μg/ml) of aqueous extract were added to the platelet suspension for 1 min exposure at 37 ºC before treatment with platelet aggregating agents. Aspirin at 500 μg/ml was used as a standard.

Antihemolytic Activity: Antihemolytic activity of the extracts was followed as described by Naim *et al*. 1976 [7]. Blood was collected from healthy adult human volunteers and erythrocytes separated by centrifuge at 1,000 g for 10 min. Obtained RBC pellets were washed with phosphate buffer (0.9% NaCl at pH 7.4) and diluted with saline to give a 4% suspension. Samples with different concentrations of extract (100, 200, 300, 400, and 500 μg/ml) were added to 2 ml of the erythrocyte suspension, and the volume was made up to 5 ml with saline buffer. The mixture was incubated at 37 ºC for 1 h at room temperature, and then 0.5 ml of H_2O_2 solution in saline buffer was added. The concentration of H_2O_2 was adjusted to bring about 90% hemolysis of the blood cells. After incubation, the reaction mixture was centrifuged at 1,000 g for 10 min, and the color density of the supernatant was measured by absorbance at 540 nm. The percent of hemolysis inhibition was calculated using the formula:

Inhibition percentage = Abs control - Abs sample / Abs control × 100

RESULTS AND DISCUSSION:

Antiplatelet Activity: The process of hemostasis is the activation of platelets that are stimulated by the result of platelet aggregation due to complex signal transduction cascade reactions [8]. Thus, platelets accelerate the formation of fibrin and thrombin. In many conditions, dysfunction and increased platelet reactivity contribute to the development and progression of the disease [9]. Therefore, compounds from natural sources that inhibit platelet aggregation are of great importance.

From **Fig. 1** it was evident that the hydroethanolic leaf extract of *Annona muricata* and standard aspirin was found to be effective antiplatelet

activity in a dose-dependent manner with maximum activity at 500 µg/ml concentration. However, the prevention of platelet aggregation was lower when compared to standard to aspirin at 500 µg/ml concentration. Antiplatelet drugs have important roles in preventing multiple diseases like cardiovascular, cerebrovascular, and peripheral arterial systems [10].

Although there have been a lot of reports suggested that the side effects occur by synthetic drugs like aspirin and heparin are used to treat cardiovascular diseases [11]. The flavonoids present in hydroethanolic extract might have prevented the adhesion and aggregation of platelets besides the release of cytoplasmic calcium that in turn stimulates the release of ADP. These results are in accordance with the observation made by Brindha and Arthi *et al.*, 2010 [12] who reported that both pink and white flower extracts of *Nelumba nucifera* have different levels of inhibitory action on platelets aggregation depending upon the concentration of phytochemical compounds.

Antihemolytic Activity: Reactive oxygen species (ROS) can lead to hemolysis and eventually to diseases such as thalassemia and sickle cell anemia. Their action can be counteracted by the antihemolytic activity of therapeutic agents [13]. The percentage of hemolysis inhibition by leaf extract of *Annona muricata* was found to be 64.94 ± 0.01 %, while that of standard ascorbic acid was 69.77 ± 0.03 % at 500 µg/ml concentration **Fig. 2**. It is observed that the hemolysis of leaf extract was higher (333.76 ± 0.012 µg/ml) than those of the standard ascorbic acid (316.09 ± 0.08 µg/ml), which may be due to the ability of the leaf extract to stabilize the erythrocyte membrane.

In several studies, hemolysis had been used in measuring free radical damage and its antioxidant inhibition by performing erythrocytes in blood. This test is useful for screening studies on different molecules and their metabolites, particularly those with an oxidizing or antioxidizing activity, and molecules with a long-term effect [14]. Our findings are in par with the results observed by Omale *et al.*, 2014 [15,] who reported that *Gymnema Sylvestre* extracts exhibited concentration-dependent inhibitory activity against hydrogen peroxide-induced hemolysis of erythrocytes.

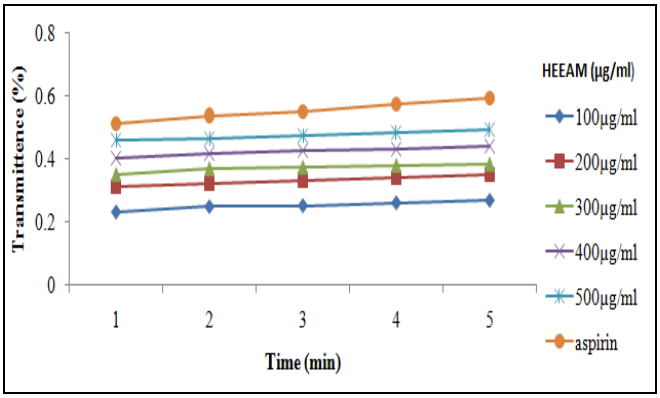

FIG. 1: EFFECT OF ANTIPLATELET ACTIVITY OF ANNONA MURICATA LEAVES EXTRACT. Values are Mean ± Sd

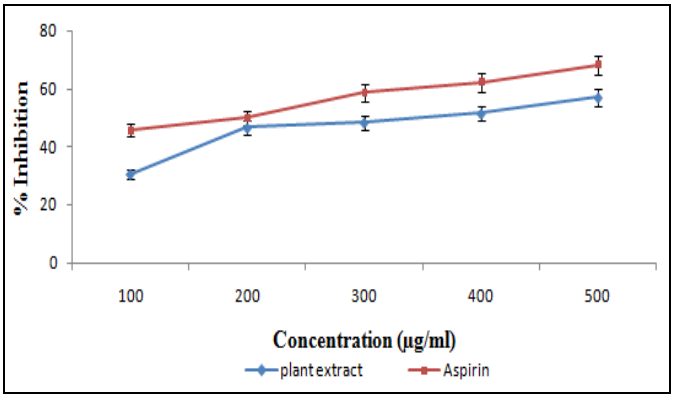

FIG. 2: EFFECT OF ANTIHEMOLYTIC ACTIVITY OF ANNONA MURICATA LEAVES EXTRACT. Values are Mean ± Sd

CONCLUSION: HEEAM exhibited concentration dependent inhibitory activity towards hydrogen peroxide-induced hemolysis of erythrocytes, which is attributed to the bioactive constituents, which exert a protective effect against oxidative injury against biological macromolecules in the erythrocyte membrane. In the present study, the higher antihemolytic potential was exhibited by HEEAM due to the presence of high phytochemical content in the extract. From the results of the study, it was concluded that the traditional medicinal plant leaves, *Annona muricata* possessed a considerable level of antiplatelet and antihemolytic activities. Therefore, it is concluded that this plant leaves can be used as a potential source of medicinal drugs.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

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