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Screening of Anti-Inflammatory and Anti-Platelet Aggregation Property Studies from *Ipomea staphylina*

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Abstract

Inflammation is a biological reaction which is a part of non-specific immune response that occurs in a series of reaction to any type of injury, damage caused to the body. Although there has been enough progress in the usage of colossal improved anti-inflammatory drugs, they are known to make up just around half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which might affect the central nervous system. Thus researches in screening up of anti-inflammatory components from the plants have been shown very good progress. Such similar property was been screened using *Ipomea staphylina*, a local weed belonging to Convulvulaceae family. Study included the mechanism by which the Convulvulaceae species I. staphylina inhibited inflammatory pathway key enzymes. Plant extract was randomly divided into 3 groups namely water extract, ethanol extract and methanol extract. We screened by randomized trials to estimate levels of all the three key enzymes activity involved in inflammation i.e., PLA2, COX (cyclooxygenase) & LOX (lipoxygenase) using the plant sample.

Results show that the water extract of the Plant sample inhibited secretory PLA2 significantly than the other groups. PLA2 was significantly inhibited as the concentration of the sample increased and this showed reproducible results. COX and LOX had inhibitory effects but not to a greater extent. These data suggest that the crude plant water extract had molecules that inhibited PLA2 which is the first enzyme in the inflammatory pathway.

Keywords: Anti-inflammatory; Anti platelet aggregation; *Ipomea staphylina*; Phospholipase A2; Plant aqueous extract; COX; LOX

Introduction

The potential of higher plants as source of pharmacologically important has not been much explored. The plants though have great potential in medicinal field, most of the species are yet to be discovered and most of the ones which are identified have not yet submitted to the phytochemical investigation. Thus lots of plant mysteries are open for the researchers to explore and isolate the potential active bio-components which are medically important. One of such important plant genus was Ipomea which had great medical values [1-7]. As we all know the second largest genus of Convulvulaceae family is Ipomea with around 600 species [8]. Ipomea species are usually found in tropic or subtropical regions which are exclusively known for their therapeutic values such as Anti-inflammatory, alkaloids are known to have shown potential anticancer properties. *Ipomea staphylina* is one of the recently identified species of Convulvulaceae family which are infected by *Caviceps purpurea*, leading to the presence of ergot alkaloids in them [9-11]. Bioactive components like low molecular weight phenolic compounds and Flavones are widely distributed among the Ipomea genus. Flavones are known to possess potential biochemical effects which inhibit variety of enzymes such as xanthine oxidase, lipoxegenase, etc. [12].

The beneficial effects of the Flavones are known by mechanisms that include antioxidant, anti-inflammatory, anti thrombic, anti-adhesive, anti-vasodilatory and anti-tumor properties too [13-16]. To understand its role in inflammatory pathway, it is important to understand the inflammatory mediators. As we all know that Infammation is the biological response hosted by the body against any harmful stimuli or foreign visitors (pathogens) or damage to the cells [17,18]. The phases of inflammation could be divided into several phases based on the time period. The inflammatory mediators are usually categorized into exogenous and endogenous mediators. Exogenous mediators are usually bacterial toxins. Endogenous mediators are again categorized into early phase mediators which include histamine, serotonin, chemo-attractants etc., that are produced by mast cells and platelets and late phase mediators include lipoxygenase, leukotrienes and cycoloxygenase [19,20].

sPLA2 (secretory Phospholipase A2) is known to catalyze the first step in the Arachidonic acid pathway which is keynote substrate for the enzymes Lipoxygenase and cycloxgenase [21-24]. sPLA2 are abundantly present in mammalian pancreatic secretion and in snake venom. $sPLA_2$ acts mostly on the phosphotidyl choline of the membrane lipids which are abundant in the cell membrane at C2 position giving rise to a lysophospholipid and Arachidonic acid [25,26]. This Arachidonic acid serves as substrate for COX and LOX enzymes based on whether it is taking cyclic (COX) or linear (LOX) pathway. If COX enzyme acts on the substrate then Prostaglandins are formed. Based on whether it is COX 1 or COX 2 enzyme that acts on Prostaglandin, the products formed are Prostacyclins, PGD2 (Prostaglandin D2), PGE2 (Prostaglandin E2) and Thromboxanes respectively. Prostacyclins are known to cause vasodilation and inhibit platelet aggregation. Thromboxanes have counter effect unlike Prostacyclins. They are known to cause vasoconstriction and promote platelet aggregation (Figure 1).

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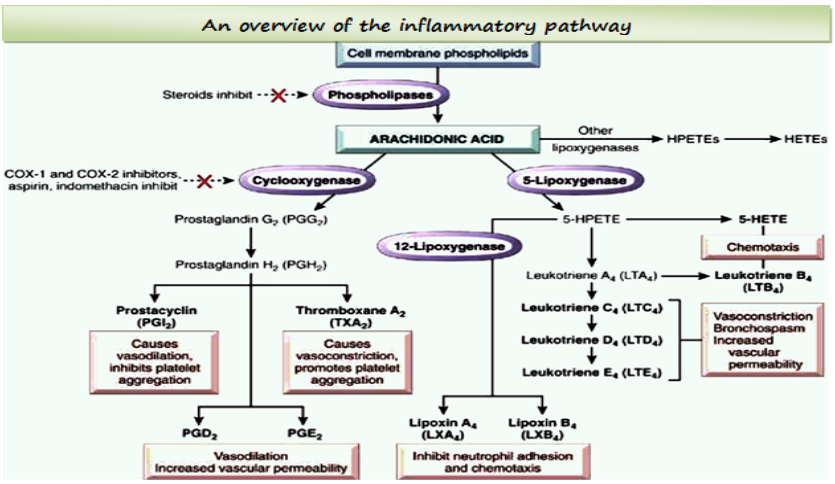

Figure 1: This figure illustrates the pictorial overview of the inflammatory pathway.

Arachidonic acid forms various HPETEs (hydro-peroxy-eicosa-tetraenoic acid) like 5, 12, 15 HPETEs by 5, 12, 15 Lipoxygenases respectively [27-32]. 5 HPETEs will form leukotrienes which enhance vasoconstriction, Bronchospasm and increased vascular permeability. 12, 15 LOX will form Lipoxins and Hepoxins respectively where Lipoxins are known to have chemotactic effect and inhibit neutrophil adhesion whereas Hepoxins function still remains unexplored. In this study we have screened the anti-inflammatory and antiplatelet aggregation properties from *Ipomea staphylina*.

Materials and Methods

Preparation of plant extract and protein estimation of snake venom

Known amount of dried leaves of *Ipomea staphylina* were taken with little amount of water and centrifuged at 1500 rpm for 15 minutes at 4°C. Supernatent obtained was used for further experiments. Similar procedure was carried out for solvent extraction using 50% ethanol which was used for Indirect Hemolytic Assay.

Concentration of protein present in snake venom (*Daboia russelii* (Russel viper)) was estimated using BSA as standard (1-75 µg) by Lowry's method [33].

Indirect hemolytic assay

Erythrocytes were isolated from fresh blood taken from the donor and it was added to 3% Trisodium citrate (9:1 v/v) by centrifuging at 1500 rpm for 15 min at room temperature by using Phosphate buffer saline until clear supernatant was obtained. The pellet was used for further study.

Indirect Hemolytic Assay was carried out using PLA2 standard as described by Boman and Kaletta in 1957 [34]. One ml of erythrocytes was taken to which 1 ml of egg yolk and PBS was added in the ratio of 1:8. One ml of this suspension was added into different concentration of venom and incubated for 30 minutes at 37°C. This reaction mixture was stopped by adding ice cold PBS and centrifuged at 1500 rpm, 4°C for 10 min. The supernatant was measured colorimetrically at 540 nm.

Determination of platelet aggregation activity

Ardlie and Han method was employed to isolate platelets from fresh human donor [35]. Turbidometric method was employed to follow platelet aggregation where agonist platelet aggregating agent was added to platelet rich plasma the platelets aggregate and gradually becomes clear increasing light transmission. The change in turbidity is observed and plotted on a recorder.

Polyphenols and flavones identification

Estimation of total polyphenols content was carried out by Folin-Ciocalteu method using gallic acid as standard. Similarly Flavones were also estimated by Woisky & Salatino Colorimetric method using Quercetin as standard.

These were further supported by Concentrated Sulphuric acid test and Mg-HCl test.

Malonyl dialdehyde assay

Known amount was plasma was added to blank, control and sample tubes to which known amount of BHT and TCA was added and centrifuged at 10,000 rpm for 20 min followed by incubation in boiling water bath for 30 min. Absorbance was read at 532 nm spectrophotometrically.

Lipoxygenase assay

Isolation of PMNLs of human blood was carried out by Ficoll-Histopaque density gradient and hypotonic lysis of erythrocytes. These PMNLs were re suspended in PBS and sonicated at 20-30s at 20 kHz to release the cytosolic 5 LO enzyme into the solution. This solution was centrifuged at 100,000g for 30 min at 4°C. The supernatant is directly used as source of enzyme. 5 lipoxygenase enzymatic assays was carried out using buffer, DTT, ATP and CaCl2 and the end product HETE was measured at 234 nm using Shimadzu spectrophotometer.

Results and Discussion

Indirect Hemolytic Assay showed significant result. Plant aqueous extract showed almost 90% of PLA2 inhibition (Figures 2 and 3). Thus this implies that the plant is a potent inhibitor of phospholipase A2

which also implies its important role in inhibiting the first step in inflammatory pathway.

However plant extract did not show any significant effect in Platelet aggregation activity. This could be viewed by the recorder plotted graph (Figure 4). This was further confirmed by Malonyl Dialdehyde Assay.

The estimation of polyphenols and flavones showed erect graph which indicates its presence, (Figures 5 and 6). This was further supported by conc. sulphuric acid test. Mg HCl test clearly produced pink to orange effervescence see (Figure 7).

However plant extract decreased the activity of 5 Lipoxygenase enzymes significantly. This might be helpful in improving the endothelial function which has role in decreasing coronary heart disease. This also has significant role in other medical therapeutics [36-39] (Figure 8).

Conclusion

Phospholipases A2 superfamily plays a crucial role in the biological system by extending diverse cellular responses including phospholipid digestion. *Ipomea staphylina* aqueous extract are a potent biological compound capable of efficiently inhibiting PLA2 enzyme. However the biological conditions matter in its variability of the result in the inhibition. The extract did not show much significant effect on platelet aggregation which was further confirmed by Malonyl Dialdehyde assay. The plant extract however contained the medically important compounds that possessed diverse health benefits such as Flavonoid and polyphenols. The molecules were not exactly identified, thus further studies have to be carried out for the purification of the active ingredient and their effects on animal model. If the crude extract is capable of exhibiting such beneficial effects then the isolated molecules might show much significant result.

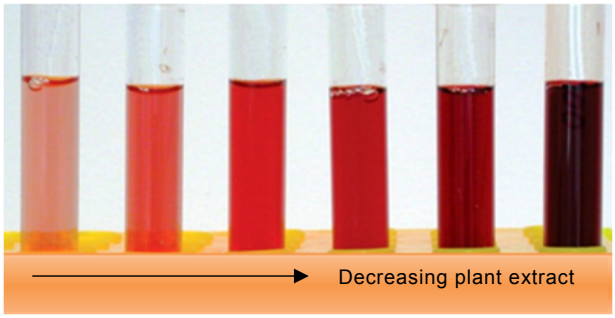

Figure 2: This figure shows clearly the inhibition of the PLA2 in first test tube decreasing gradually as the plant aqueous extract concentration decreased.

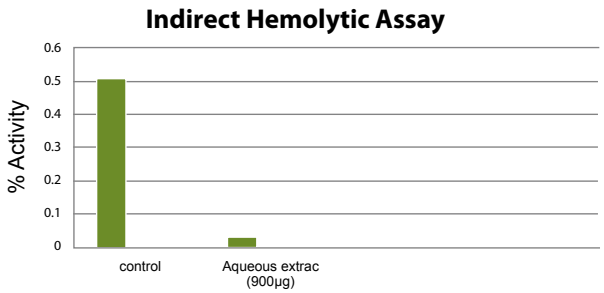

Figure 3: Indirect Hemolytic Assay showing almost 90% inhibition of PLA2 by plant aqueous extract.

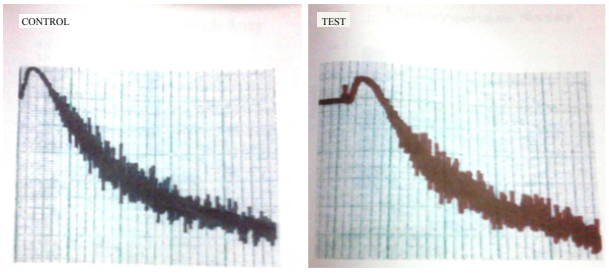

Figure 4: The graphs do not show any significant effect of plant extract on Platelet aggregation activity.

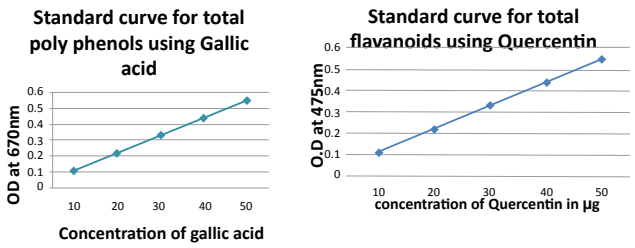

Figure 5,6: These both graphs represent the presence of Polyphenols and Flavanoids in the plant aqueous extract.

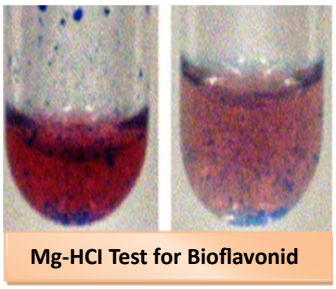

Figure 7: Mg-HCl Test to identify the presence of Flavonoid in the diluted plant extract.

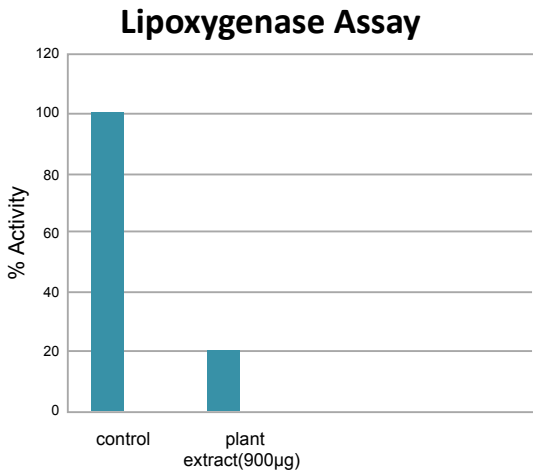

Figure 8: Graphical representation of Lipoxygenase Assay. This shows that the plant aqueous extract significantly reduced the activity of the enzyme 5 LOX.

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