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Membrane stabilizing activity – a possible Mechanism of action for the anti-inflammatory Activity of *Psidium Guajava* in rats

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ABSTRACT

In an effort to scientifically appraise the mechanism of action of *Psdium guajava* stem bark and leaves, the present study was carried out to investigate the cell membrane stabilizing anti-inflammatory activities of methanol extracts of psidum guajava on experimental animal model at three different dose levels – 5mg/kg, 10mg/kg and 15mg/kg. Aspirin (10mg/kg) was used as standard reference cell membrane stabilizing agent for comparison. This research work was carried out using Wistar strain albino rats weighing 150g-175g. The extractive inhibited heat-induced haemolysis of erythrocytes in vitro. The methanol extract of *Psidum guajava* stem- bark demonstrated 89.23%, 93.94% and 103.66% inhibition while the leave extract demonstrated *51.40%, 51.43%, 61.40% and 44.29%* of hemolysis of RBC caused by heat, in a dose- dependent manner – 5mg/kg, 10mg/kg and 15mg/kg respectively. Results were considered significant at $p \leq 0.05$.

Keyword: *Psidium guajava,* membrane stability, albino Wistar rats, hemolysis, erythrocytes.

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INTRODUCTION:

Before the availability of synthetic drugs man was completely dependent on natural medicinal plants (herbs) for curing diseases. Herbs become more popular in recent years due to public awareness and increasing interest among consumers and scientific communities. Guava like many other herbs has an amazing qualities and it seem to do amazing things. It is able to stops inflammation, diarrhea, scurvy and malaria[1] and it has saved many lives across the world especially in impoverished countries and in regions where modern health care is either not accessible or not affordable or both.

Guava also known as *Psidium guajava* is a global plant, belonging to a family of myrtacease. It is a small tree that grows up to 35 feet tall, it is easy to recognize because of its smooth, thin, copper- colored bark and also because of the attractive "bony" aspect of its trunk. It is a native to and widely distributed in Mexico and Central America. However, the plant is cultivated today from the west coast of Africa to the pacific region including India and China.[2]From preliminary medical research in laboratory setting extracts from guava bark or leaves are implicated in possible therapeutic mechanism against inflammation, bacterial infections and pain [3,4]

Epidemiological evidence has revealed that constituents in guava products such as flavonoids (guercetin, morin, rutin and kaempferol), polyphenol, anthraquinones etc show many biological and pharmacological activities.[5] Flavonoids are known to have various effects on mammalian cellular systems and structures and have been shown to protect biological membranes against free radical induced oxidative damage and inflammation[6] Kaempferol, rutin and quercetin have also displayed an array of other pharmacological features such as decreasing capillary permeability, fragility and inflammation [7] and inhibition of hemolysis [6]Natural medicine derived from guava extracts had being increasingly utilized to treat a wide variety of clinical diseases, although relatively little knowledge about their mode of action is available. In an effort to scientifically appraise the mechanism of action of anti-inflammatory activity of *Psdium guajava* stem – bark, the present study is carried out to investigate the possible cell membrane stabilization of anti-inflammatory activities of *Psidum guajava* stem- bark methanol extract in Wistar rats.

MATERIAL AND METHODS

Plant material

The experimental plant, Guava stem bark and leaves were collected in the month of July, 2011 from Okoroma community in Oyigbo Local Government Area, River State, and South- South, Nigeria and were properly identified using normal standards.

Experimental Animals

Forty albino Wistar rats weighing between 150g – 175g of were used for this study. (Twenty rats in each group). The animals were purchased from University of Nigeria animal house, Nsukka. The animals were housed in the pre-clinical animal house, college of medicine, University of Port Harcourt, Port Harcourt, Nigeria, under standard laboratory conditions of temperature (25-270c), humidity and light (12 h light and 12h dark cycle), and were allowed to acclimatize for 3 weeks and were fed with standard pellet and water given ad libitum.

Method of Study

The experimental protocols and procedures used in this study were carried out according to the guideline in research and teaching stipulated by the institutional Animal Ethical committee (IAEC). (University of Port Harcourt, Nigeria)

Extraction

The plant (guava stem bark and leave) was sun dried for several days and then oven dried for several hours at considerably low temperature (not move than 35ºc) and reduced to powdery form using an electric blender. The powdered material (400g) of each was extracted with 500ml of 50% aqueous methanol in the cold for 72h. The methanol extract was evaporated to dryness using rotator evaporator under reduced pressure at 40ºc and a yield of 85.3g (21.3%) was obtain, and it was then stored in refrigerator.

Effect of the extract on membrane stability.

Blood sample was collected from rats into EDTA bottles to prevent clotting. Isotonic buffer (5ml) containing varying concentration of the extract (5mg/kg, 10mg/kg, and 15mg/kg) and aspirin (10mg/kg) were put into two duplicates sets of centrifuge tubes [8]. The same volume (5ml) of the isotonic buffer was added into another tubes containing concentration of aspirin (10mg/kg) as control. Erythrocyte suspension (30µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54ºc for 20 min in a water bath. The other pair was maintained at 0 - 5ºc in an ice bath. The reaction mixture was centrifuge for 3 min at 1300g and the absorbance of the supernatant was read at 540nM Wave length using spectrophotometer. The percentage hemolysis inhibition or membrane stability in the test was determined according to the equation.

$$\% \text{ stabilizing activity} = 100 \times 1 - \left[\frac{(OD_2 - OD_1)}{OD_3 - OD_1}\right]$$

Where,

OD1 = test sample unheated

OD2 = test sample heated

OD3 = control sample heated

Statistical Analysis:-

Values are expressed as mean + SEM. Statistical significance was determined using two–way ANOVA test. Values with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

In this in-vitro experiment carried out using rat blood, the membrane stabilizing activity of the extracts increased significantly in a dose-dependent manner (5mg/kg, 10mg/kg and 15mg/kg) compared with Aspirin (10mg/kg). Results were considered significant at $P \leq 0.05$.

The tables 1 and 2 of the % membrane stability in the present study showed clearly that hemolysis decreased in a dose-dependent manner in the presence of the guava extracts. The results showed that 5mg/kg, 10mg/kg and 15 mg/kg of *Psidium guajava* extracts and 10mg/kg of aspirin stabilized cell membrane by 89.23%, 93.94%, 103.66% and 85.92% respectively for the stem bark while by 51.40%, 51.43%, 61.40% and 44.29% respectively for the leave. This indicated that *Psidium guajava* extract could be more effective than aspirin in this study.

Table 1:Effect of Psidium Guajava Stem bark extract on red blood cell membrane

Stem bark	Concentration (mg/kg)	% membrane stability
Psiduim guajava	5	89.2± 0.006
Psiduim juajava	10	93.94±0.006
Psidium guajava	15	103.66± 0.006
Aspirin (control)	10	85.92± 0.006

Table 2: Table showing % membrane stability of the leaf extract relative to aspirin

Leaf extract	Concentration(mg/kg)	% membrane stability±SEM
P. guajava	5.0	51.4 ± 0.006
P. guajava	10.0	51.43 ± 0.006
P. guajava	15.0	61.40 ± 0.006
Control(aspirin)	10.0	44.29 ± 0.006

Furthermore, no destruction or lytic effects of cell membrane were observed, which could have occurred as a result of cell membrane destabilization[9]. This probably indicated the membrane stabilizing action of the extracts. The study, also demonstrated capability of the extracts to stabilize red blood cell membrane, which is an indication of the extracts' ability to prevent rupture or hemolysis in heat-induced stress condition.

However, the exact mechanism of action, responsible for the membrane stabilizing activity of the plant extract, could not be established in this study, and also the chemical constituent (s) of the

psidium guajava stem bark extract that might be responsible for the observed membrane stabilizing action was not identified with certainty. Nevertheless, Flavonoids present in guava extract has various effects on mammalian cellular system and structures and has shown to protect biological membrane against free radical-induced oxidative damage and inflammation[6]

Sequel to this, a number of investigators has shown that, flavonoids, and a host of other secondary plant metabolites, exhibited anti-inflammatory effects as a result of their membrane stabilizing action in various experimental animal models[10,11,12,13]

Furthermore, it has also been reported that, there is production of free radicals, such as lipid peroxide and Superoxide in various conditions, such as heat-induced stress hemolysis, due to cell membrane destabilization[14] Flavonoids and other phenolic compounds are good scavengers of free radicals due to their antioxidant properties [15,16,17,18] Since the studied plant extracts have been reported to contain flavonoids [19,20] The quercetin, morin and keempferol increased membrane protein which play vital role in preserving cell membrane stability [6,22] Flavonoid also raises the level of reduced glutathione that directly protects membrane proteins and preserved their stability. Decrease levels of glutathione result in oxidization of membrane protein and loss of membrane stability. Flavonoid (rutin) has also displayed an array of other pharmacological features such as decreasing capillary permeability (thereby reducing edema), fragility and inflammation[7]. The flavonoid inhibits cyclo-oxygenase-2 (that increase formation of prostaglandins), lipoxygenase (which increase formation of leukotrienes) and prostaglandin hydroperoxidase activity (which was not affected by non-steroidal anti-inflammatory drugs (NSAIDs)[22] and this result to increase in the stability of the cell membrane.

Pure quercetin had also been showed to inhibit hemolysis by 35.5% at the highest concentration (10µg / ml), ranking third among the flavonoids tested for hemolysis inhibition[6] It is not unreasonable, therefore to speculate that flavonoids and other chemical components in the plant extract are responsible for the observed membrane stabilizing action, and probably as a result of their action as free radicals scavengers, inhibition of cyclo-oxygenase-2 (leaving cyclo-oxysenase-1 pathway alone, which has beneficial effect) and inhibition of lipoxygenase, Serotonin and histamine (potent inflammatory mediators) and increase in the level of reduced glutathione that directly protects membrane proteins and preserved their stability.

CONCLUSION

The experimental evidence obtained in this present laboratory animal study indicates that, the extracts of *Psidium guajava* possess membrane stabilizing property which was speculated to be

playing a significant role in its anti-inflammatory activity. *Psidium guajava* stem bark and leave extracts could serve as a useful supplementary therapy in hemolytic disease, and also in free radical mediated oxidative cell injury conditions.

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