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Psidium Guajava's Effect on Acute Phase Protein Levels during Acute Inflammation.

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ABSTRACT

In spite of wide spread biological uses of *Psidium guajava*, there is a dearth of information on its hepatoprotective activity especially during inflammation. This work was therefore conducted to evaluate the effects of methanolic stem bark and leave extracts of the plant on acute-phase proteins during acute-phase response in rats. Forty albino Wistar rats (twenty in each group) were divided into two groups (stem bark and leave extracts). Inflammation was induced using egg albumin while treatment with the extracts commenced as soon as the inflammation was established and this lasted for 90 minutes. Initial, inflammation and recovery phase blood samples were obtained for analysis of acute-phase proteins (Albumin & C-reactive protein) using standard methods. Even though the stem bark extract showed more potent effects on both parameters in either dose-dependent and time-dependent fashions, both were perpetuating their anti-inflammatory potency through significant reduction on C-RP and increment on Albumin levels purporting a possible mechanism of action for anti-inflammatory activity of *Psidium guajava*. Results were considered significant at $P \leq 0.05$.

Keyword: *Psidium guajava*, C-reactive protein, Albumin, acute-phase response, anti-inflammatory, egg albumin, albino Wistar rats.

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INTRODUCTION:

P. guajava has a rich ethno medicinal history. Different parts of the plant are used in various indigenous systems of medicine, primarily for the treatment of G.I ailments[1]. In Africa and other parts of the world, people use natural resources for medicinal purposes. *Psidium guajava* (guava) is a medicinal plant used in tropical and sub-tropical countries to treat many disorders such as diarrhea, cough and gastrointestinal disorders. It was reported that Psidium guajava leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis[2, 1], and antidiarrhoeal and narcortic properties[3]. Guavas are plants in the Myrtle family (Myrtaceae). Genus = Psidium (meaning "pome granate" in Latin) [4, 5] which contains more than 100 species of tropical shrubs and small trees. They are native to Mexico, Central America and Northern South America. The leaf or stem extract is used to treat diarrhea, abdominal pain, convulsions, epilepsy, cholera, insomnia and also has hypnotic effect.[6]

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response).

In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL-1, IL-6 and IL-8, and TNF-α.

Positive acute-phase proteins serve different physiological functions for the immune system. Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, Mannose-binding protein, complement factors, ferritin, ceruloplasmin, Serum amyloid A and haptoglobin while negative acute-phase proteins decrease in inflammation. Examples include albumin, transferrin, transthyretin[7] retinol-binding protein, antithrombin, transcortin. The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is generally to provide amino acids in order to produce "positive" acute-phase proteins more efficiently. Theoretically, a decrease in transferrin could additionally be decreased by an upregulation of transferrin receptors, but the latter do not appear to change with inflammation.[8]

C - Reactive protein (CRP) and Albumin are acute phase proteins. The latter was originally named for its ability to precipitate the C-polysaccharide of Pneumococcus in the presence of calcium [9].It is the prototypic acute phase reactant whose presence in plasma or serum serves as a useful laboratory indicator of systemic inflammatory disease. Normally, CRP in human biological fluids is present in trace amounts (0.07-8mg/L, median 0.6mg/L) stimulated by certain

cytokines (IL-1 α , IL-1 β, TNF- α and β and indirectly by IL-6)[10,11] During the acute phase response, CRP's serum concentration can increase up to 1000-fold within a few hours while the albumin behaves the other way round. Among acute phase proteins, CRP is a fast-reacting, sensitive, and the most easily measured one with a rapid response time, short half life and large incremental change and its catabolism is not affected by the type of inflammation so then, can be used as an early and pre-clinical marker of Inflammation.

Inflammation is a complex, highly orchestrated process. It involves many cell types and molecules, some of which initiate, amplify, or sustain the process, some of which attenuate tissue injury by modulating it, and some of which cause it to resolve. A number of APPs have the potential to influence one or another stages of the inflammatory response.CRP, a component of the innate immune system,[12] has been presumed to play a significant role in the clearance of infectious agents, as well as damaged cells, through its ability to bind to phosphocholine.CRP can activate the classical complement pathway when bound to one of its ligands and can also bind to phagocytic cells, suggesting that it can initiate elimination of targeted cells or infectious organisms by interacting with both humoral and cellular immunity. Moreover, CRP can participate in the inflammatory response by inducing production of inflammatory cytokines[13] and tissue factor.

This research work was therefore carried out in order to evaluate the activities of guava leaves and stem bark extracts on acute-phase protein levels in inflammation in albino Wistar rats as one of the possible mechanisms through which *Psidium guajava* perpetuates its anti-inflammatory activity.

MATERIALS AND METHODS

Plant Material:

The plant specimens (stem barks and leaves) used were collected in the month of July, 2011 from Okoroma in Oyigbo L.G.A Rivers State, Nigeria and were air-dried at Laboratory temperature 25-27c and reduced to powdery form using an electric blender; from which the methanolic extracts of the stem bark and leaves were prepared separately.

Animals

Forty albino Wistar albino rats weighing between 125—200g of mixed sex used for this study were breed and housed in the pre-clinical animal house, college of Medicine, University of Port Harcourt, Nigeria. The animals were kept and maintained under Laboratory conditions of temperature, humidity and light; and were allowed free access to food (standard pellet) and

drinking water ad- Libitum. The experimental protocols and procedures used in this study were approved by Ethical Committee, University of Port Harcourt, Rivers State, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH Publication No. 85-93, revised 1985).

Extraction:

The powdered material (400g of each specimen) was extracted with 500ml of aqueous Methanol in the cold for 72h. The Methanol extract was evaporated to dryness using rotary evaporator under reduced pressure at 40c and a yield of 85.3g (21.3%) was obtained. Phytochemical screening of the extracts was done as described[14].

Induction of Inflammation:

Egg white-induced paw oedema was used for evaluating the anti-inflammatory activity of the extract, 20 rats weighing between 125-200g were randomly distributed into four groups (n=4).Before inflammation was induced, the volume of the right hind paw was measured using plethysmometer in the two separate sets of experiment using the two extracts.

Acute inflammation was produced by injecting a fresh egg albumin (0.2ml of 50% solution) into the plantar surface of the rat right hind paw according to a modified method [15]. The test extracts were administered at different doses (5mg/kg, 10mg/kg, and 15mg/kg) and the standard drug Aspirin (200mg/kg) was used as a control. Finally, after about 20-30 minutes, the blood samples of the rats were collected into EDTA bottles for analysis.

The twenty rats in each group were divided into four groups of five animals each. Group 1 which served as control received 20% aspirin at 10ml/kg, while group2, 3, 4, received the extract intra-peritoneally at the doses of 5, 10, 100 mg/kg respectively, of which the blood samples were collected at interval for analysis.

Blood analysis.

CRP and albumin ELISA kits were purchased from Abazyme, LLC Needham, MA USA and were used for the quantitative determination of C reactive protein and albumin concentrations in serum according to manufacturer' instructions and standard methods.

Statistical Analysis

The results were tabulated as mean ± SEM. The data were analyzed using Analysis of Variance with multiple comparism, POST HOC (LSD, Duncan, Turkey, and Scheffe). The differences were considered at significant $p< 0.05$. The program used was Statistical Package for Social Sciences (SPSS) version 17.

RESULTS AND DISCUSSION

The previous studies have demonstrated that during inflammation, some acute phase proteins increase, while some of them decrease.[16]

Those proteins that that are elevated are referred to as *positive* acute phase proteins (e.g. C-Reactive peptide (C-RP), haptoglobulin, a1-acid glycoprotein etc), while those that decreased are referred to as negative acute phase proteins (e.g. albumin, transferrin, transthyretin etc). It has also been shown that C-RP can participate in the inflammatory response by inducing production of inflammatory cytokines[13] However, it is clear that monocytes and macrophases at the site of inflammation constitute the major source of these cytokines, particularly IL-6, IL-1b and TNFa. IL-6 is considered to be the major inducer of APP (acute phase protein) gene expression, since it, either alone or by enhancing the effects of other cytokines, inducing virtually all APPs[12]

In this research work, it was extrapolated that when the inflammation was induced, the level of the CRP increased, while the level of albumin decreased, relative to the induced inflammation. The increased level of CRP may be based on the fact that CRP can play a role as modulator of the inflammatory response[17]. CRP, a component of the innate immune system, has been presumed to play a significant role in the clearance of infection agents, as well as damaged cells, through its ability to bind to phosphocholine. This may probably be the reason why the CRP level increases during inflammation[13]. Also CRP can activate the classical complement pathway when bound to one of its ligands and can also bind to phagocytic cells, suggesting that it can initiate elimination of infectious organisms by interacting with humoral and cellular immunity.

Tables 1 and 2 showed decrease in the volume of inflammation within 90 minutes, which was dose-dependent. When *Psidium guajava* leaf extract and aspirin were administered, the leaf extract reduced the volume of the paw and it was dose-dependent. 5mg/kg of the extract showed 1.77% decreased in volume, while 10mg/kg and 15mg/kg of the leaf extract showed 4.20% and 5.66% decrease respectively. But when 15mg/kg of aspirin is used, it showed that the volume of the paw decreased by 6.50% within 90 minutes, likewise, when the stem extract (5mg/kg, 10mg/kg, 15mg/kg) was administered the level of paw volume reduced by (52%,8.3% and 13.3%) respectively, thus re-establishing the anti-inflammatory activities of the plant[18]

Comparing the overall effects of both extracts in reducing inflammation, the stem bark extract seemed to be more potent. It also showed that aspirin is more efficacious in reducing inflammation than the same concentration of guava extracts in this short-term analysis.

Figures 1 and 2 showed the levels of albumin before, after inflammation and when the inflammation had been treated. Aspirin served as the control in relation to the *Psidium guajava* extracts. Both extracts significantly($p \leq 0.05$) reversed the negative effect of the inflammation nevertheless, comparing the same concentration of aspirin and the extracts, it showed that aspirin increased the level of albumin than the extract, thus has more efficacious in treating inflammation.

Table1. Effect of *Psidium guajava* bark extract and aspirin on egg albumin-induced acute-inflammation in rat's paw.

Treatment Groups	Dose(mg/kg)	Initial paw volume (ml±SEM)	Mean change in paw volume(ml)			% oedema volume relative to the control after 1:30mins
			Paw volume (30mins) (ml±SEM)	Paw volume (60mins) (ml±SEM)	Paw volume (90mins) (ml±SEM)	
Control	_	0.59±0.12	1.28±0.01	1.27±0.12	1.27±0.11	
Aspirin	15	0.67±0.11	1.05±0.01	0.90±0.12*	0.79±0.11*	82.4
Psidium	5	0.68±0.12	1.22±0.11	1.15±0.02*	1.11±0.02*	36.7
guajava	10	0.62±0.11	1.01±0.12	1.00±0.01*	0.92±0.12*	55.9
	15	0.65±0.01	1.07±0.01	1.02±0.12*	1.00±0.11*	48.5

All values are expressed as mean ± SEM (n=5) *p<0.05

Table2. Effect of psidium guajava stem bark extract and aspirin on egg albumin-induced rat paw oedema

Group	Dose (mg/kg)	Initial Volume (ml)±SEM	Volume After 30mins (ml±SEM)	Volume After 60mins (ml±SEM)	Volume After 90mins (ml±SEM)	% decrease in paw volume after treatment
Control	-	0.98±0.01	1.39±0.11	1.38±0.02	1.37±0.01	
Aspirin	*15*	0.85±0.01	1.86±0.10	1.42±0.11*	1.32±0.11*	20.5
Extract	*5*	0.91±0.11	1.85±0.10	1.55±0.12*	1.37±0.10*	17.9
	10	0.96±0.10	1.55±0.10	1.49±0.01*	1.47±0.10*	30.8
	15	0.98±0.02	1.66±0.12	1.54±0.11*	1.53±0.11*	44.4

Results presented in ± SEM. P<0.05.

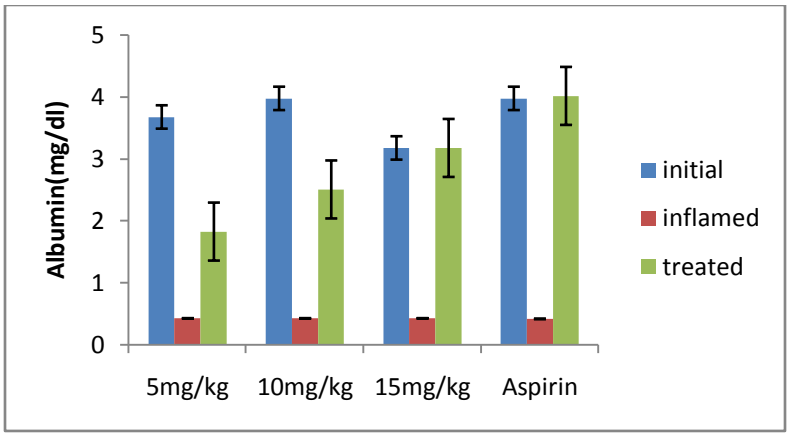

Figure: 1 Effect of *P. Guajava* leaf on albumin during inflammation

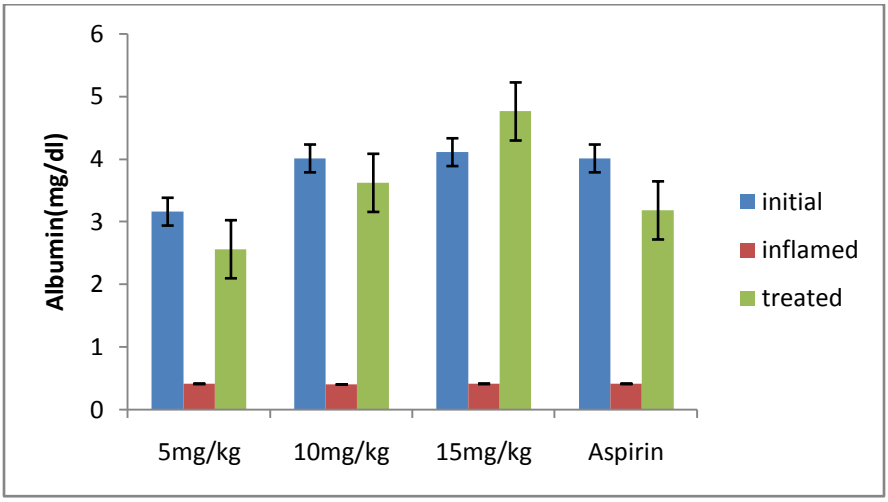

Figure 2: Effect of *P. Guajava* stem bark on albumin during inflammation

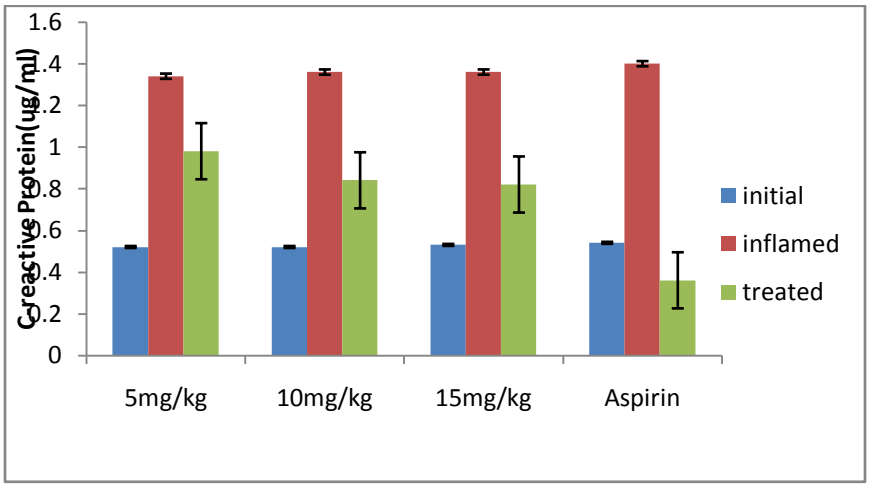

Figure 3: Effect of *P. Guajava* leaf on CRP during inflammation

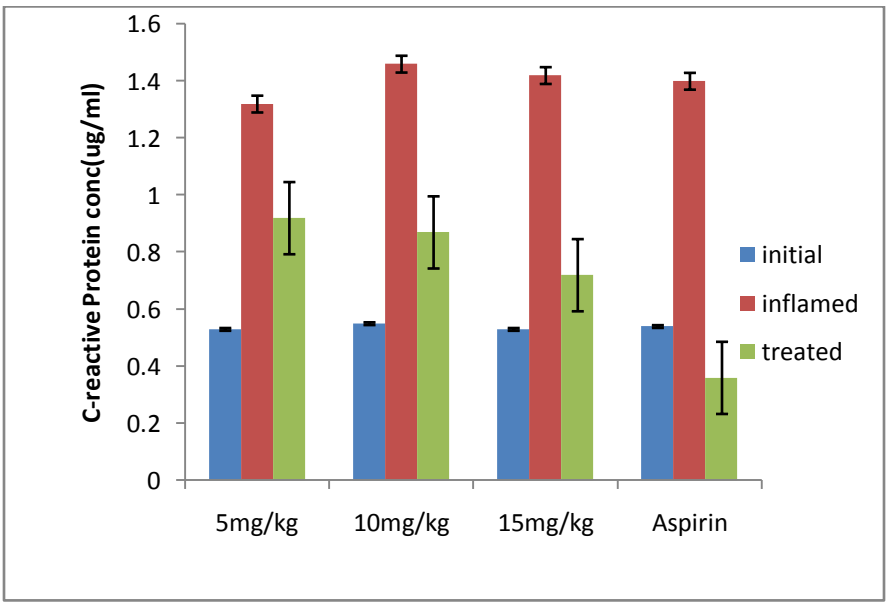

Figure 4: Effect of *P. Guajava* stem bark on C - reactive protein during inflammation

Figure 3 and 4 showed the patterns of level of C-RP before inflammation, after inflammation and when the inflammation has been treated. The CRP levels were seen to be lowered by both extracts significantly ($p \leq 0.05$) and both could achieve this feat by dose- and time-dependent fashion.

Comparing the same concentrations of stem bark and the leaf extracts, it could be categorically stated and showed that the former is more efficacious in decreasing the CRP level, thus can reduce the volume of the paw much faster than the latter in a dose-dependent manner.

However, in summary, when both guava extracts were administered, the level of C-RP decreased, while the albumin level on the other hand increased, both of which occurred in dose dependent manner. This probably explains the anti-inflammatory effect of the guava extracts, and possibly one of the mechanisms of action since it decreases the level of C-RP, which plays role as modulator of inflammation response[17] The extracts also increased the level of albumin, contrary to the decreased level when the inflammation was induced.

It is therefore speculated that the chemical constituents of the *psidium guajava* specimens might be responsible for the observed decreased level of CRP and increased level of albumin Nevertheless, a number of investigations has speculated that flavonoid and other constituents of guava leaf are responsible for this anti-inflammatory effect, portrayed by decreased level of CRP However, recent studies, particularly studies of transgenic mice over expressing CRP, indicate that CRP can also display anti-inflammatory effects[19]. Such effects may be at least partly explained by the ability of CRP to prevent neutrophil adhesion to endothelial cells by decreasing surface expression of L-selectin[20], to inhibit superoxide anion generation by neutrophils and to induce synthesis of IL-1Ra by mononuclear cells and increased level of albumin[21, 22, 23], thus inhibiting inflammation.

CONCLUSION

From the experimental data gathered from this research work, it can be deduced that the *psidium guajava* leaf and stem bark extracts could be perpetuating their established anti-inflammatory influence through their modulation on various levels of acute-phase proteins known to either increase or decrease by 25% during inflammation.

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