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Hematological Activities of Psdium Guajava During Acute Inflammation In Rats

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ABSTRACT

This research work evaluated the effects of *Psidium guajava* leaf extract on differential white blood cell count, % Packed Cell Volume(PCV) and Hemoglobin (Hb) concentration in acute - induced inflamed wistar rats. The study involved the use of forty five rats (in two groups) and were each divided into various subgroups (5mg/kg, 10mg/kg, 15mg/kg of extract and aspirin and control groups), with average weight of 150-270g. The initial, inflamed, and treated values of rats paw volumes were taken and after inducing inflammation with egg albumin and after a period of administration of the extract and aspirin, the rats were sacrificed and blood samples were collected. The results obtained at the end of the experiment showed that there were differences in values (i.e. initial, inflamed and treated). There was an increase in %PCV, WBC, Hb concentration after administration of extract and aspirin which led to a decrease in inflammatory effect. The effect may be due to the anti-inflammatory components present in *P. guajava* leaf and stem bark extracts which not only inhibit the production of inflammatory mediators but equally have multiplier effects on acutely low hematologic parameters. Based on these results, it is clear that *P. guajava* extracts possess components that sustain prompt recovery of these hematologic parameters during inflammation, a possible mechanism of action of its anti-inflammatory effect on animals.

Keywords: *Psidium guajava*, anti-inflammatory, %PCV, WBC, Hb concentration, egg albumin.

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INTRODUCTION:

The long history of guava use has led modern day researchers to study guava extracts. Its traditional use against diarrhea, gastroenteritis and other digestive complaints has been validated in numerous clinical studies; antioxidant activity[1] CNS activity[2] anti-diabetic activity3 antimicrobial activity[4,5], hepatoprotective activity[6], treatment of plague[7], spermatoprotective activivty[8].

In a study including medical plants on anti-proliferative effects on human mouth epidermal carcinoma and murine leukemia cells using MIT assay, guava leaf showed anti-proliferative activity, which was 4.37 times more than vincristine[9] . Bark and leaf extracts were shown to have in vitro toxic action against bacteria. Gallocatedrin isolated from the methanol extract of guava leaf showed antimutagenic activity against E.coli . The antimicrobial activities of P.guajava and leaf extracts, determined by disk diffusion method (zone of inhibition) were compared to tea tree oil (TTO), deoxycydine and clindamycine antibiotics. It was shown that P.guajava leaf extract might be beneficial in treating acne, especially those that have anti- inflammatory activities[10]. The active flavonoid compound-quercetin-3-0-alpha-1-arabinopy ranoside (guajaverin) extracted from leaves has high protein antiplague activity by inhibiting the growth of streptococcus aureaus in a study carried out by disc diffusion method[4] . A double-blind clinical study of the effects of a phytodrug (QG-5) developed from guava leaf showed a decrease in duration of abdominal pain which is attributed to anti spasmodic effect of guercetin present in the leaf extract[11]. The microbicidal activity of *psidium guajava* is attribtuted to guajaverine and to psydiolic acid[12] . Anti-inflammatory and analgesic activities of *P. guajava* have been reported [13]. Here, we evaluated the activities of *P.guajava leave and* stem bark extracts on packed cell volume, white blood cell count and hemoglobin concentration during acute inflammation in laboratory rats.

MATERIALS ANS METHODS

Plant Material:

Plant samples were collected in the month of August, 2011 from Okolomo in Afam/Oyigbo Local Government Area of Rivers State in the Southern part of Nigeria, where voucher specimen was deposited with number 106998. The plant was air-dried at laboratory temperature 25-27^{O}C and reduced to powdery form using an electric blender.

Animals

Forty five albino wistar rats weighing between 150-270 g of mixed sex were used for this study; they were bred and housed in the Basic Medical Sciences (BMS) animal house, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria. The animals were kept and maintained under laboratory conditions of temperature, humidity and light; and were allowed free access to food (standard pellet diet) and drinking water *ad libitum.*

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Port Harcourt, Rivers State, Nigeria and conformed to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

Extraction

The powdered material was extracted with 500 ml of 50% aqueous methanol in the cold for 72h. The methanol extract was evaporated to dryness using rotary evaporator under reduced pressure at 400C and a yield of 85.3 g (21.3 %) was obtained. Phytochemical screening of the extract was done as described[14].

Induction of Inflammation:

Egg white-induced paw oedema was used for evaluating the anti-inflammatory activity of the extract, 25 rats weighing between 150-270g were randomly distributed into four groups (n=5).Before inflammation was induced, the volume of the right hind paw was measured using plethysmometer by Hugo Basale(Italy)

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 extract was administered at different doses (5mg/kg, 10mg/kg, and 15mg/kg) and the standard drug Aspirin (10mg/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 five rats were divided into five groups of five animals each. Group 1 which served as control I received 10ml distilled water, group II received aspirin at 10mg/kg, while group2, 3, 4, received extract intraperitoneally at the doses of 5, 10, 15 mg/kg respectively, of which the blood samples were collected at interval for analysis. Finally, the paw volumes of the rats' right hind paw was measured at 30 minutes, 1 hour, and 1 hour30minutes. The inhibitory activity was calculated according to the following formula *[16]*:

$$\text{Percentage inhibition} = \frac{(C_t - C_o)\ \text{control} - (C_t - C_o)\ \text{treated}}{(C_t - C_o)\ \text{control}} \times \frac{100}{1}$$

Where C_t is the mean change in paw volume (in ml) at time t, C_o is the mean change in paw volume (in ml) before egg albumin injection. (C_t-C_o) control, is oedema or mean change in paw

size after egg albumin injection to control rats at time t. (C_t - C_o) treated, is oedema or mean change in paw size after egg albumin injection to treated (reference or extract) rats at time t.

Effect of extract on hematologic parameters:

Twenty rats were divided into four groups of five animals each. Group 4 which served as control received 10mg/kg of aspirin while groups 1, 2, 3,which served as test group received extract intra-peritonially at the doses of 5, 10, 15 mg/kg respectively for 30mins. Post inflammation. The doses chosen were based on the median lethal dose (LD50) obtained, Blood samples (2 ml) were collected each from rats of all groups, 60mins after treatment for assessment of the hematological parameters.

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 & DISCUSSIONS

Previous researches have shown that during inflammation, the levels of hemoglobin concentration, percentage packed cell volume and white blood cell decrease[16] *and* the reason for this may be based on the fact that during inflammation, the liver produces large amount of hepcidin, which in turn stops ferroportin from releasing iron stores, thus reducing the hemoglobin concentration during inflammation[17] *while* studies on some medicinal plant extracts have reported significant decrease in PCV, Hb and WBC, when administered orally to Wistar rats for five days and this decrease was attributed to the haemolysis of the blood cells, as a result of cell membrane destabilization by the extracts [18,19]

However, in this present study, it was obviously observed from the results,(tables 1 and 2 revalidated the anti-inflammatory activities of the extracts even during acute inflammation and at relatively small dosage levels 5, 10, and 15mg/kg) that after inducing inflammation, the hemoglobin concentration, percentage packed cell volume and white blood cell count decreased, but when treated with the guava extracts, (5 mg/kg, 10mg/kg and 15mg/kg) the above mentioned parameters began to increase and the degree of this increase was dose-dependent. Interestingly, no destruction or lytic effect of administered plant's extract were observed on any blood cells in rats, which could have occurred as a result of cell membrane destabilization.[20]

Table 1:Effect of *Psidium guajava* leaf 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*	20.5
Psidium	5	0.68±0.12	1.22±0.11	1.15±0.02*	1.11±0.02*	17.9
guajava	10	0.62±0.11	1.01±0.12	1.00±0.01*	0.92±0.12*	30.8
	15	0.65±0.01	1.07±0.01	1.02±0.12*	1.00±0.11*	44.4

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

Table 2: 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

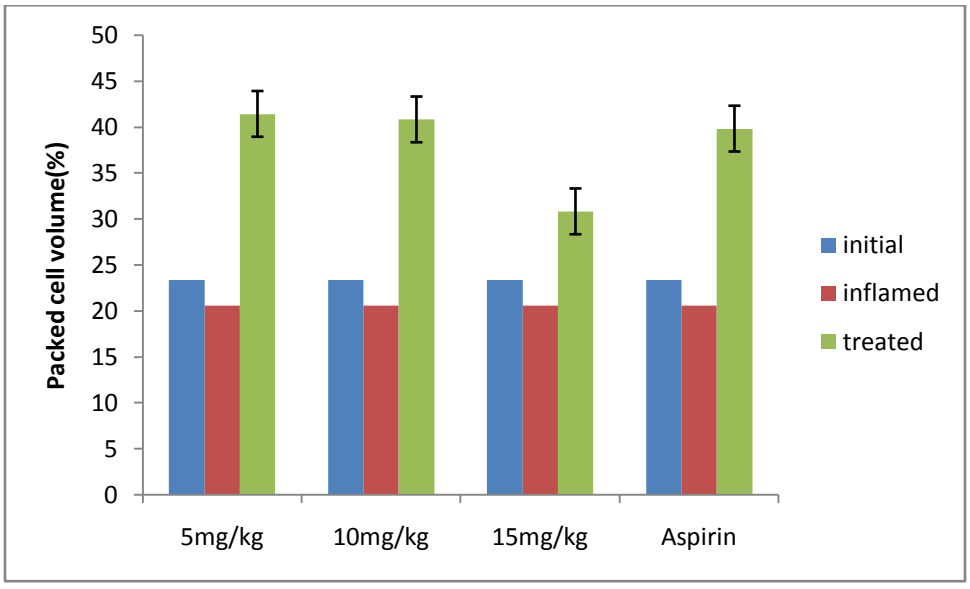

Figure 1 a: Effect of P.guajava leaf extract on packed cell volume during inflammation

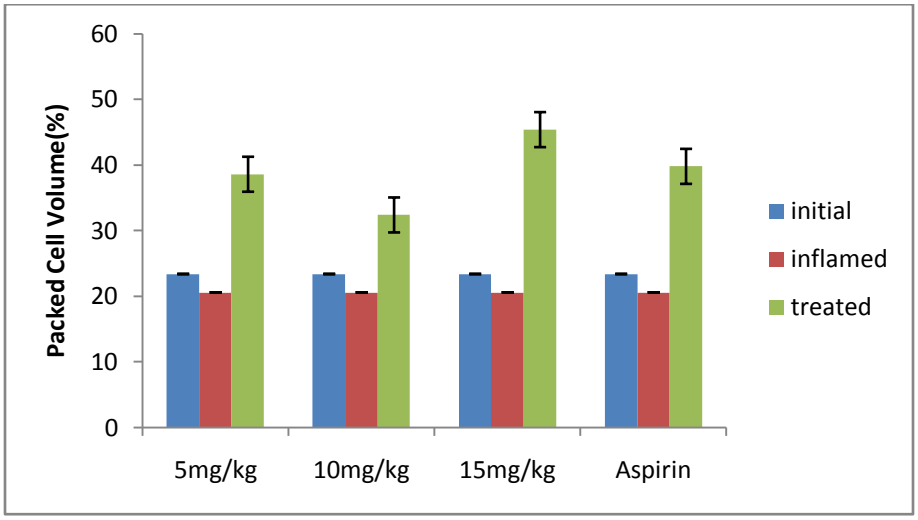

Figure 1 b: Effect of P.guajava stem bark on packed cell volume during inflammation

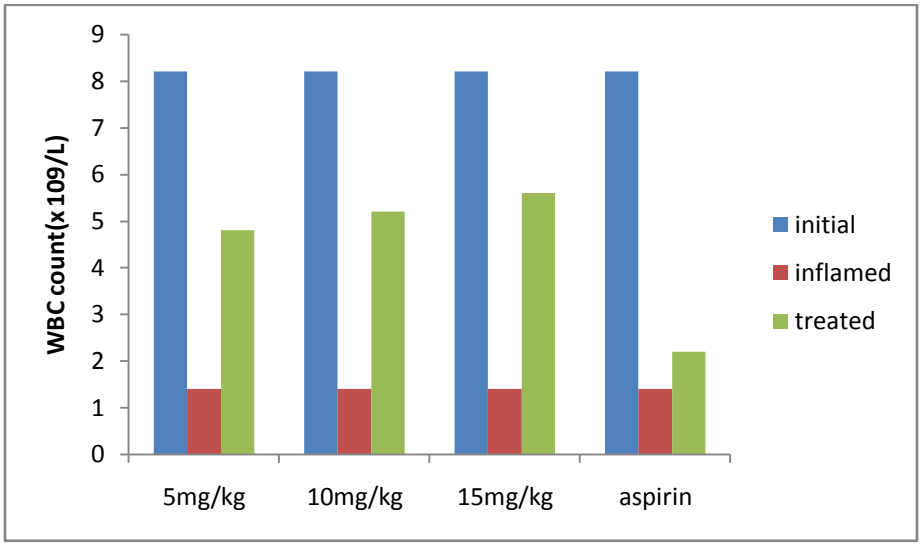

Figure 2 a: Effect of P.guajava leaf extract on WBC counting during inflammation

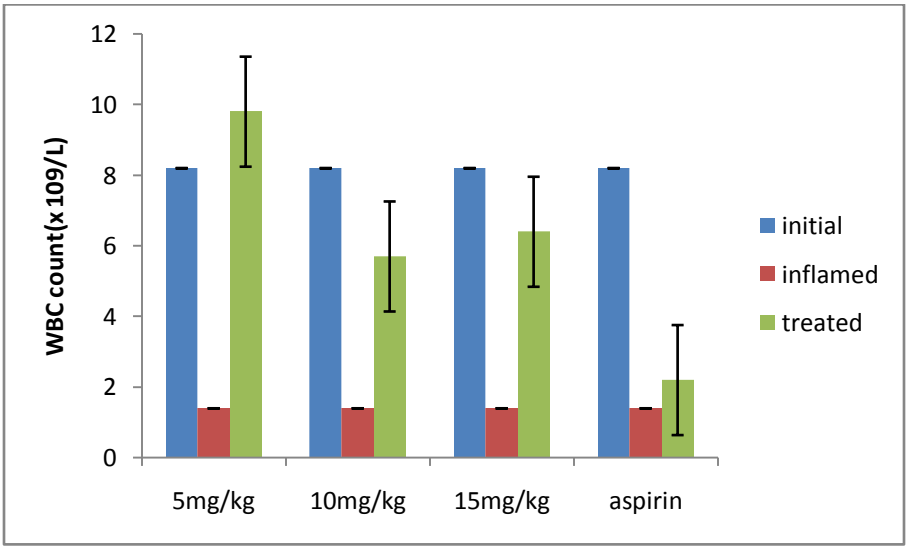

Figure 2 b: Effect of P.guajava stem bark on WBC during inflammation

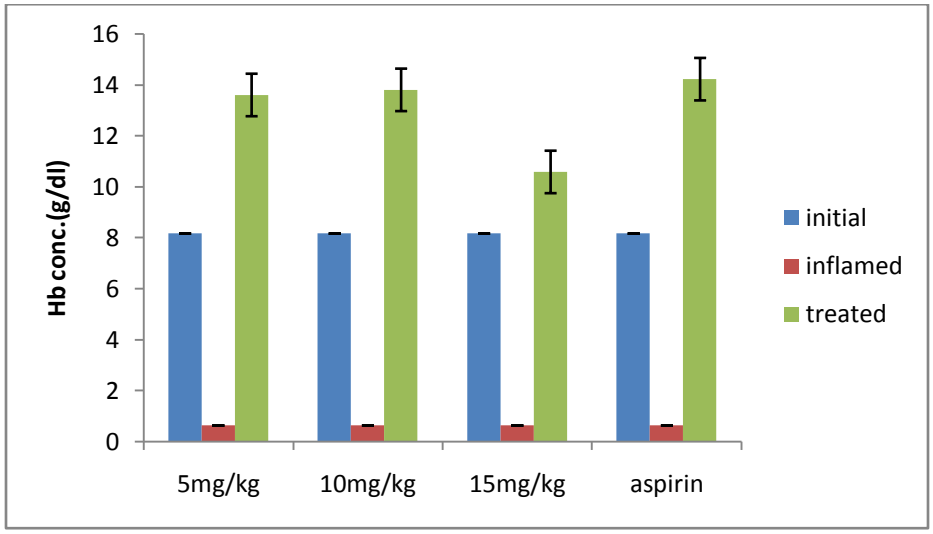

Figure 3 a: Effect of P.guajava leaf extract on hemoglobin concentration during inflammation

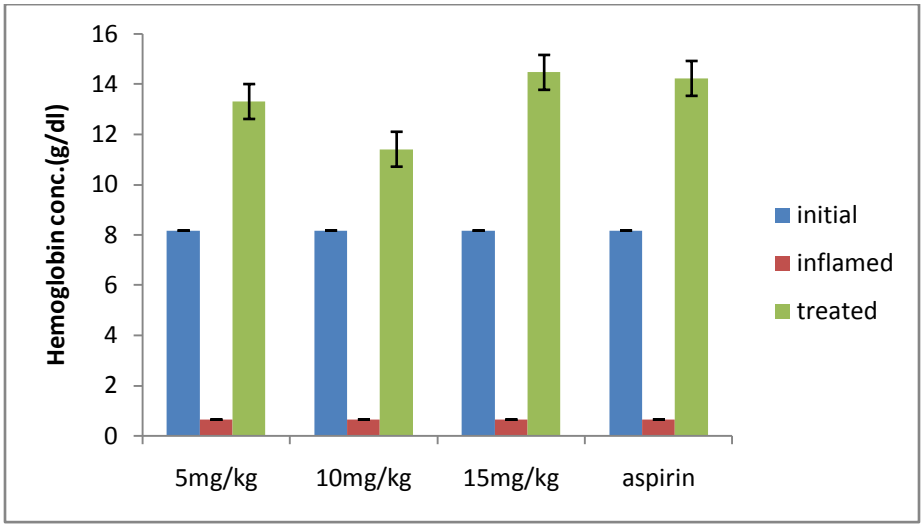

Figure 3 b: Effect of P.guajava stem bark on hemoglobin concentration during inflammation

Comparing aspirin as a reference point with the guava leaf extract, it was shown from the table that the guava leaf extract is more efficacious in increasing the percentage packed cell volume and white blood cell count than aspirin, but on the other hand, aspirin was more efficacious in increasing the hemoglobin concentration than guava leaf extract.

Comparatively, the activity of the leave extract was dose-dependent on white blood cell while it was not so about the stem bark, however, the effect of the latter was greater in boosting the level of white blood cells (figures 2a and b). On hemoglobin concentration, there was a significant increase during the recovery phase though not dose-dependent however, aspirin group showed more positive response while the stem bark was much better when compared (figures 3a and b). On the packed cell volume, the leave extract's effect was lowest at the highest dose but with an

overall positive activity while at highest dose using stem bark extract, the effect was at its peak showing the potency of the extract in hematology(figures 1a and b).

This increment in the level of these parameters by the leaf and stem bark extracts may probably be that the extracts has the ability to inhibit the production of hepcidin, which in turn increases the production of ferroportin, thus increasing the iron store[21] and equally has established hepatoprotective activity[6] . In a study of aqueous extract of P. guajava in acute experimental liver injury induced by carbon tetrachloride, paracetamol and thioacetamide, it showed hepatoprotective activity. The effects observed were compared with a known hepatoprotective agent, silymarin. Histological examination of the liver tissues supported hepatoprotection [6] with positive effect on bones. The increased iron store will then increase the hemoglobin concentration and also percentage packed cell volume level**.**

CONCLUSION

The experimental evidence obtained in the present laboratory animal study indicates that, the aqueous-methanol extract of *P.guajava* possesses membrane stabilizing property. *P. guajava* extract could serve as a useful supplementary therapy in hemolytic disease, and also in free radical-mediated oxidative cell injury conditions.

REFERENCES

1. Wang B, Jiao S, Liu H, Hong J. Study on Infected with Corynebacterium-pseudotuberculosis. J Comp Path 2004; 84: 477-484. 159: 4514-4523.
2. Ticzon and Roneo, Ticzon Herbal Medicine Encyclopedia. Romeo R. Ticzon Publishing, 1997; ISBN No. 97191-7231
3. Mukhtar HM, Ansari SH, Bhat ZA, Naved T, Singh P. Ant diabetic activity of an ethanol extract obtained from the stem bark of Psidium guajava. Pharmazie 2006; 61(8):725-727.
4. *Abdelrahim SI, Almagboul AZ, Omer MEA. Antimicrobial Activity of Psidium Guajava. Fitoterapia. 2002; 73(7-8):713-715.*
5. Arima H. Danno G. Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. Bioscience, Biotechnology and Biochemistry. 2002; 66(8):1727-1730.
6. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. Ind J Exp Biol 2006; 44: 305-311.
7. Prabu GR, Gnanamani A, Sadulla S, Guaijavenn. A. Plant flavonoid as potential antiplaque agent against Streptococcus Mutans. J Appi Microbiol, 2006; 101(2): 487-95.

8. *Akinola OB, Oladosu OS, Dosumu OO. Ethanol extract of the leaves of Psidium guajava Enhance Sperm output in healthy Wistar rats. Afr J Med Sci, 2007; 36(2): 137-140.*
9. Manosroi, J, P. Dhumtanom and A. Manosroi. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett 2005; 235: 114-120.
10. Qadan F, Thewaini AJ, Ali DA, Afifi R, Elkhawad A, Matalka KZ. The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne- developing organisms. Am J Chin Med 2005; 33,197-204.
11. Xavier Lozoya, Hortesia Reyes, Soto-Actions on DU145 cells. *2010; eCAM*., 1- 8.
12. Berdy JA, Aszalos M, Bostian, Mcntt KL. Handbook of antibacterial compounds.1981; Bocaratom, CRC press.
13. Olorunfemi OJ, Nwafor A, Nworah DC**.** *Psidium guajava* effects on the febrile sponses to baker's yeast injection in rats**.** Int J Pharma Sci 2011; 3(2):1407-1412.
14. Trease E, Evans WC. Textbook of Pharmacognosy. Oxford University. Press, Oxford. 1989; 13th Edn:m512-513.
15. Winter CA, Risely EA, Nuss, GW. Carrageen -Induced Oedema in hindpaw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med 1962;111:544-5.
16. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr 2006; **26** (1): 323–42.
17. Vecchi C, Montosi G, Zhang K, *et al*. ER stress controls iron metabolism through induction of hepcidin. Science 2009; **325** (5942): 877–80.
18. Wu. Tianshung, Chan Yuyi, Liou, Maeijen, Fulwen, Shilishan, Chen Kutung. Platelet aggregation inhibitor from *Murraya euchrestifolia*. In: Proceeding of 2ed international symposium on natural drugs, Maretea Italy, 1997; 28 September- Oct 1.
19. Plotnikov MB, Koltuniv AA, Aliev OI, Kalinkina GI, Berezovskaya TP, Andreeva, VTU, Tikhonova LA. The haemorrheological properties of extracts from some plants containing flavonoids. Rastitel Nye Resury 1998; 34:87-91.
20. Hess SM, Milong RC. Assay for anti-inflammatory drugs. In: Lepow, IH, Ward, PA, (Eds) Inflammation Mechanisms and Control. Academic Press New York 1972; 1-2.
21. Knutson MD, Oukka M, Koss LM, Aydemir F, Wessling-Resmick M. Iron release from macrophage after erythrophagocytosis is up-regulated by ferroportin1 over expression and down-regulated by hepcidin. Proc Natl Acad Sci USA; 2005; 102:1324-8.