

# EFFECTS OF *PSIDIUM GUAJAVA* AQUEOUS EXTRACTS ON HAEMATOLOGICAL PROFILE AND SERUM LIPID VARIABLES OF ALBINO RATS

ARUNA ROSHNI KULLU, WAZIDA TABASSUM AND M. P. SINHA\*

Department of Zoology, Ranchi University, Ranchi- 834008, Jharkhand.

e-mail: m\_psinha@yahoo.com

## KEYWORDS

*Psidium guajava*,  
haematological, serum  
lipid

## Received on :

03.03.2013

## Accepted on :

15.05.2013

\*Corresponding  
author

## ABSTRACT

The effect of aqueous extract of leaves on some haematological and serum lipid parameters in rats during a seven day administration of the doses of 250mg/kg and 500 mg/kg body weight orally was investigated. The parameters evaluated include serum lipids, red and white blood cell indices. The results show that the extract administered significantly increased ( $p < 0.05$ ) packed cell volume, haemoglobin concentration, red blood cell, MCH, MCHC, MCV and platelet count at the dose of 250mg /kg and 500 mg/kg body weight when compared with control. Whereas *Psidium guajava* the platelet was significantly increased ( $p < 0.05$ ) at 250mg/kg body weight but at 500mg/kg body weight the count significantly reduced ( $p < 0.05$ ). Also, the extract significantly increased ( $p < 0.05$ ) white blood cell count at all doses administered when compared with control. Moreover, the extract significantly reduced ( $p < 0.05$ ) total cholesterol concentration, triglycerides and HDL-cholesterol concentration in the serum while it had no significant effect on serum LDL-cholesterol concentration at all doses administered when compared with controls. The results of this study suggest that the extract may have beneficial effect on serum cholesterol concentration and triglycerides reduction as well as in anemia and immunity dependent disorders.

## INTRODUCTION

Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology (Borchers *et al.*, 2000). Plant extracts are potentially curative. Some of these extracts can boost the humoral (Rehman *et al.*, 1999) and cell mediated immunity (Upadhyay *et al.*, 1992) against viruses (Calixto *et al.*, 1998), bacteria (Boyanova and Neshev, 1999), fungi (Ali *et al.*, 1999), protozoa (Sharma *et al.*, 1998) and cancer (Wong *et al.*, 1994).

Guava (*Psidium guajava* Linn.), belonging to the Family Myrtaceae, is originated in the tropical South America (Pathak and Ojha, 1993) and grows wild in Bangladesh, India, Thailand, Brazil, Florida, West Indies, California and also in several other countries (Bailey, 1960). The main constituents of guava are vitamins, tanins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. These and other compounds are related to many health effects of guava (Haida *et al.*, 2011). Some authors have found high concentrations of carotenoids (beta-carotene, lycopene, and beta-cryptoxanthin), vitamin C and polyphenols in guava pulp (Oliveira Dda *et al.*, 2010; Ordóñez-Santos and Vázquez-Riscos, 2010; Ramírez and Delahaye, 2011). Lycopene has been correlated with the prevention of cardiovascular damage because of its positive effects on dyslipidemia (Lorenz *et al.*, 2012; Sesso *et al.*, 2012).

Cardiovascular diseases causes some of the main health problems, major ones are coronary heart diseases, stroke and hypertension (Alters and Shiff, 1997) and elevated plasma

lipids are risk factors in cardiovascular problems ( Yakabu and Afolayan, 2008). Hyperlipidaemia and other abnormal blood lipid profile are largely of genetic origin or due to unwholesome nutritional habits. Lipids and other substances accumulate on arterial wall, forming plaque, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver, or kidney. Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure, as the case may be ( Gabriel *et al.*, 2008).

It has also been reported that hypercholesterolemia is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction which are common causes of morbidity and mortality (Crowell, 2004 and Owolabi *et al.*, 2010). Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the reduction of lipid profile is considered to be an important therapeutic approach and efforts have been made to identify the lipid lowering effect of various medicinal plants (Onat *et al.*, 2006). The prevention of oxidation of low density lipoprotein cholesterol by the antioxidant compounds like poly phenolics and flavonoids is also important in the prevention of cardiovascular diseases and these phytochemicals are present in plants and plant products which are helpful in treating various other diseases (Choudhury *et al.*, 2012 a,b; 2013 a,b,c).

The blood is a vital fluid, which contains the Red Blood Cell (RBC), White blood cells (WBC) and platelets suspended in the serum in homeostatic concentrations. The Blood is important for pulmonary and tissue respiration, as a medium

of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion (Adebayo et al., 2005), nutritional and immunological processes, as well as homeostatic responses (Oze, 1992). The laboratory determination of blood products and parameters for the purpose of disease diagnosis is highly accurate, sensitive and reliable; and remained the bed-rock of ethical and rational research, disease diagnosis; prevention and treatment (Murrey et al., 2000, Okonkwo et. al., 2004).

Despite of several studies on the different pharmacological activities of *P. guajava*, not much has been investigated on interrelationship of lipid and haematological profile. Therefore the present study is aimed to investigate the impact of leaf extract of *P. guajava* on lipid profile and haematological indices in mammalian animal model.

## MATERIALS AND METHODS

**Collection of plant material:** The fresh and tender leaves were collected, dried in a shade under  $28 \pm 2^\circ\text{C}$  (for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

**Extract preparation:** 50 g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using  $\sim 350$  mL distilled water. The extract obtained was filtered, concentrated in rotary flash evaporator and maintained at  $45^\circ\text{C}$  the percentage yield of each extract were calculated and the dried extracts were stored in air tight containers at room temperature for further studies.

**Animals:** Albino rats (175-200 g) were used in the study. They were maintained under standard laboratory conditions at ambient temperature of  $25 \pm 2^\circ\text{C}$  and  $50 \pm 15\%$  relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet and water *ad libitum*. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Ranchi University, Ranchi.

### Experimental design

The animals were randomly assigned into three groups of six rats each as follows:

Group 1: Received 1mL of distilled water orally

Group 2: Received 250mg/kg b w body weight of *P. guajava* orally.

Group 3: Received 500mg/kg body weight of *P. guajava* orally.

### Sample Collection

By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g (Wisperfuge, 1384, Samson, Holland) for 5 minutes. The supernatant was used for the lipid analysis. The remaining blood sample was put in an EDTA bottles for haematological determinations.

## ANALYTICAL PROCEDURE

Estimation of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was done by cholesterol oxidase - phenol aminoantipyrine method (Rifa and Warnick, 2006). The haemoglobin (Hb) level was measured by the cyanomethaemoglobin method. The Red Blood Cell (RBC) and Reticulocyte counts were determined by visual method (Baker et al., 1998). Packed cell volume (PCV) was measured using microhaematocrit method and total White Blood Cell (WBC) count was estimated by visual method (Cheesbrough, 2000). The RBC indices were calculated from the RBC count, Hb level and PCV estimations (Baker et al., 1998, Cheesbrough, 2000).

## STATISTICAL ANALYSIS

All results were expressed as mean  $\pm$  standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's-test.  $p < 0.05$  was considered as statistically significant.

## RESULTS AND DISCUSSION

The effect of the oral administration of aqueous extract of *P. guajava* leaves on some serum lipid indices is presented in Table 1. The extract significantly reduced ( $p < 0.05$ ) serum total cholesterol concentration while it had no significant effect ( $p > 0.05$ ) on serum HDL-cholesterol concentration at all doses administered when compared with control. However, the extract significantly decreased ( $p < 0.05$ ) serum triacylglycerol concentration at the dose of 250 mg/kg as well as 500 mg/kg body ( $p > 0.05$ ) it when compared with control.

The aqueous extract of *P. guajava* had significant effect on RBC, Hb, MCHC, MCH, PCV, MCV, neutrophils, basophils, monocytes, lymphocytes and eosinophils. The WBC was significantly elevated ( $p < 0.05$ ) in the group treated with 250 mg/kg body where as the count significantly decreased ( $p < 0.05$ ) in 500mg/kg body weight. The platelet also significantly increased ( $p < 0.05$ ) in rats treated with both the doses (Table 2).

High blood cholesterol concentration is one of the important risk factors for cardiovascular disease (Adebayo et al., 2005). Thus the reduction in serum total cholesterol concentration effected by the extract is beneficial and may reduce the risk of cardiovascular disease because agents that have the ability to lower cholesterol concentration in the blood have been reported to reduce vascular resistance by improving endothelial function (Adebayo et al., 2005). Similar alterations in lipid as well as haematological profiles were reported in various other plant extracts such as *Bulbine natalensis* (Yakabu and Afloyayan, 2012), *Bougainvillea spectabilis* leaves (Adebayo et al., 2005) and *Fadogia agrestis* stem (Yakabu et al., 2007).

**Table 1: Effect of aqueous leaf extract of *P. guajava* on lipid profile of albino wistar rats.**

Parameters	Group 1	Group 2	Group 3
Total Cholesterol (mg%)	60 $\pm$ 0.05	57.1 $\pm$ 0.5 <sup>a</sup>	59 $\pm$ 0.98 <sup>a</sup>
HDL cholesterol (mg%)	32 $\pm$ 0.78	30 $\pm$ 0.49 <sup>a</sup>	29 $\pm$ 0.34 <sup>a</sup>
LDL cholesterol (mg%)	20.6 $\pm$ 0.09	20 $\pm$ 0.09 <sup>ns</sup>	20 $\pm$ 0.95 <sup>ns</sup>
Triglycerides (mg%)	117 $\pm$ 2.7	113 $\pm$ 2.3 <sup>a</sup>	69 $\pm$ 1.67 <sup>a</sup>

Values are expressed as mean  $\pm$  SEM from the experiments, n=6, <sup>a</sup> $p < 0.05$ , <sup>ns</sup> = non-significant relative to control

**Table 2: Effect of aqueous leaf extract of *P. guajava* on haematological parameters in albino wistar rats.**

Parameters	Group 1	Group 2	Group 3
PCV (%)	39±0.02	42±0.01 <sup>a</sup>	44±0.02 <sup>a</sup>
MCV (%)	93±1.02	101.4±1.09 <sup>a</sup>	96.4±0.72 <sup>a</sup>
Hb (g/dL)	11.6±0.76	12±0.65	13±0.27 <sup>a</sup>
RBC (x 10 <sup>6</sup> /μL)	4.21±0.48	4.14±0.14 <sup>b</sup>	4.65±0.13 <sup>a</sup>
Platelet (x 10 <sup>3</sup> /μL)	339±1.81	365±1.09 <sup>a</sup>	305±1.57 <sup>a</sup>
WBC (x 10 <sup>3</sup> /μL)	6.8±1.62	9.1±1.57 <sup>a</sup>	8.9±0.59 <sup>a</sup>
Neutrophil (%)	56.6±1.40	62.5±0.97 <sup>a</sup>	56.4±0.87 <sup>b</sup>
Lymphocyte (%)	32.9±4.5	33.3±2.05 <sup>a</sup>	33.9±2.16 <sup>a</sup>
Monocyte (%)	5.5±1.51	5.8±2.54 <sup>a</sup>	4.9±1.94 <sup>a</sup>
Eosinophil (%)	0.5±0.07	1.2±0.08 <sup>a</sup>	4.5±0.06 <sup>a</sup>
Basophil (%)	0.28±0.09	0.29±0.04 <sup>a</sup>	0.30±0.01 <sup>a</sup>
MCH (pg)	30.46±0.35	29±1.24 <sup>a</sup>	30±0.71 <sup>a</sup>
MCHC (g/dL)	31.06±0.28	28.6±1.0 <sup>a</sup>	31±0.72 <sup>a</sup>

Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of extracts on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its products (Yakubu et al., 2007). The results obtained shows significant values of WBC, therefore it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction (Albers, 1995; George, 2000). Therefore the high dose (500 mg/kg body weight) of the *P. guajava* extracts useful in reducing the platelets which in turn might be useful in reducing the cardiovascular diseases as some studies suggested various mechanisms by which flavonoid exert its antiplatelet property by lowering intracellular Ca<sup>2+</sup> levels; alteration in the metabolism of cAMP, and thromboxane A<sub>2</sub> (Roy et al., 1999; Kang et al., 2001). The haemoglobin content, RBC and PCV has also significantly increased stimulate erythropoietin release in the kidney which is the humoral regulators of RBC production (Degruchy, 1975; Polenakovic and Sikole, 1996).

The result of this study suggested that *P. guajava* extracts studied showed positive haematological activities in rats and can be recommended in the management of anaemia and immunity dependent disorders as well as in regulating the cholesterol and triglyceride levels.

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