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Haemolytic Effect of Leaf Aqueous Extracts of Carica Papaya, Psidium Guajava and Mangifera Indica in Albino Rats

Authors
Muhammad N B, Bashiru I and Asokan C*
Department of Biochemistry, Sokoto State University, Sokoto, Sokoto State Nigeria
*Email: *asokan_74@hotmail.com; Mobile Number: +234 8025857884*

Abstract
The haemolytic effect of aqueous leaf extracts of Carica Papaya, Psidium Guajava and Mangifera Indica was investigated using albino rats. The rats were acclimatized feeding on commercial rats feed and water for a period of 20 days. The rats were than grouped in to four (4) using group 1 as controls. The remaining groups were used as subjects. The subjects were administered 4ml (20mg/kg) of the leaf aqueous extracts of the experimental plants for three (3) days. The blood sample was collected in an EDTA bottles, the blood taken was inverted slightly to ensure the EDTA mixed with blood in order to avoid clotting prior to the analysis. The packed cell volume (PCV) was analyzed using Haematocrit (Micro method). Red Blood Cell Count (RBC) was analyzed using haemocytometer method. The result obtained was expressed inform of Mean±SD student t-test and ANOVA analysis were used to compared between subjects and controls using SPSS version 20. The aqueous leaf extract of Mangifera Indica showed insignificant decrease in PCV value of the treated rats compared to the corresponding controls($P>0.05$); Also the aqueous leaf extracts of Psidium Guajava showed insignificant increase jn the PCV value of the treated rats compared to their corresponding controls ($P>0.05$).Whereas the leaf aqueous extracts of Carica Papaya showed significant decrease in the PCV value of the albino rats treated compared to their corresponding controls ($P<0.05$).The RBC values obtained in the albino rats treated showed slight decrease compared to their corresponding control but does not register any significant difference ($P<0.05$). Therefore the leaf aqueous extracts of Carica Papaya which showed significant decrease in the PCV value of the albino rats treated compared to their corresponding controls ($P<0.05$) was found to have haemolytic effects.

Key Words: *Haemolytic, PCV, RBC, Carica Papaya, Psidium Guajava, Mangifera Indica.*

Introduction

The practice of traditional medicine using medicinal plants is as old as the origin of man (G.E and W.C, 2002). It is estimated by the world health organization that, approximately 75-80% of the world's population uses plant medicines either in part or entirely (Balick *et al.*, 1996). Plants continue to be a major source of medicines, as they have been throughout human history (Shulz *et al.*, 2001). Plants constitute a major component of diet and traditional medicine in the Middle East (Mati and de Boer, 2010). It has been observed that the plants products are widely used in the treatment of various diseases in our present community. The plants products Paw-paw, Guava and Mango are widely used in the treatment of

dysentery, diabetes mellitus, typhoid fever and relief of toothache. However, many plants are reported to have serious effects, include induction of haemolytic anemia (de Freistal *et al.*, 2007). These plants though used in the treatment of various diseases, apart from therapeutic functions, may have other side effects that need to be properly taken into consideration. These side effects may include red blood cell haemolysis. To ensure their safety in treatment, there is need to screen them for such side effects. Therefore, many of the commonly used plants need to be evaluated for their potential haemolytic activity (Singh and Rajini, 2008). Red blood cells are the most common type of blood cell and are the vertebrate body's principal means of delivering oxygen from the lungs to body tissue via blood (Butler, 1975). The red blood cells can be affected by decrease or shortening of its life span which is usually about 120 days. This decrease in number of red blood cells is referred to as "anemia" (Conrad *et al.*, 2004). There are many ways or analytical tools used to diagnose this pathological condition. The commonest ones presently in use in the laboratories or haematological laboratories include red blood cells count (RBC), haemoglobin concentration (Hb), mean cell haemoglobin (MCHC) and packed volume (PCV). Packed cell volume and red blood cells count are the most common routine use to diagnose anemia in developing countries (Monica, 2008).

In this study to determine the potential and promote the use of herbal medicine, it is essential to intensify the study on the medicinal plants that are commonly in use (Garden *et al.*, 2001). This work was intended in order to investigate the haemolytic potential or otherwise of the fore mentioned plants. Previous investigation and researches have brought out their therapeutic potentials.

Materials and Methods

Sample Preparation

Sodium citrate (3.13g/dl) which is general purpose reagent produced by BDH chemical company was used to prevent clotting of red cell prior to RBC count in this experiment. Plant materials used in this research were obtained within Usmanu Danfodiyo University Sokoto and were also identified by qualified botanists in department of biological sciences.

The local name, botanical name and part of the plants to be used for the research are given below table:

SNO	Local Name	Botanical name	Parts to be used
1	Paw-paw (Gwadda)	Carica papaya	Leaves
2	Guava (gwaiba)	Psidium guajava	Leaves
3	Mango (Mangoro)	Mangifera indica	Leaves

Albino rats were obtained from the animal house of the department of biological sciences, Usmanu Danfodiyo University, Sokoto. The rats were acclimatized to the laboratory environment for a period of 20 days during which they were feed on commercial rats feed and water. The rats were than classified into four (4) groups designated I, ii, iii & iv. Each group was treated as shown below:-

Group i: Rats in this group are to serve as control. They were fed on commercial rats feed and water only.

Group ii: Rats in this group fed with commercial rats fed, water and 4ml of paw-paw leaves extracts daily for three (3) days.

Group iii: Fed on commercial rats feed, water and 4ml of the guava leaves extracts daily for three (3) days.

Group iv: Rats fed with commercial rats feed, water and 4ml of the mango leaves extracts daily for three (3) days.

Preparation of Crude Extracts

100g of leaves were put into 1000ml of water boiled and allow cooling, than 5ml of the extracts

was put into pre-weighed beaker (W1). The weight of the extract and the beaker was recorded as (W2). The content of the beaker was than deride in a drying cabinet, the content was allow to cool and its weight was recorded as (W3).

W2-W3= weight of water in 5ml of the extract (Y)

W3-W1= weight of extract (X)

Y-X= weight of dissolved solute in 5ml of the extract.

$\frac{Y-X}{5}$ = weight/ml/extract

Collection of Blood Samples

At the end of the extract administration, blood samples were obtained as describe below and used for the analysis. Chloroform was used to render the rats unconscious and the rats were than sacrificed. The blood was than collected in an EDTA bottles, the blood taken were inverted slightly to ensure adequate mixing of blood with EDTA in order to avoid clotting prior to analysis.

Determination of Packed Cell Volume (Pcv) Micro Method

The packed cell volume (PCV) can be used as a simple screening test for anemia and as a reference method for calibrating automated blood count system.

2.2-2.5ml of blood collected was put into the EDTA bottle and mixed by inversion to ensure good anticoagulation and minimize cell damage. The capillary tube was filled up to ¾ and the free end was sealed with a sealant. The tubes were then placed in the Haematocrit centrifuge and spin for 10 minutes after which the result was read using the Haematocrit reader and expressed in percent (%).

Determination of Red Blood Cell Count (Haemocytometer Method)

2.2-2.5ml of collected blood sample was put into the EDTA and mixed by inversion. 4ml of sodium citrate (3.13g/dl) was put into the test tube and 20µl (0.02ml) of the blood was added and allowed to stand for 10 minutes. The cell counting chamber (haemocytometer) was cleaned and both chamber/compartment filled with the dilute sample and covered with a cover glass, and the cells counted with the aid of tally counter using microscope at Mgx10 and Mgx40 objectives of microscope, after about 2-3 minutes. Red cells in the four (4) corners and central sub-squares was counted and the result was calculated using the formula below and expressed in trillion per litre.

$\frac{N \times DF}{A \times D} \times 106$

Where N= Number of cells counted

DF= Dilution factor (1 in 20)

A= Area of squares counted (0.02mm)

D=Depth of the chamber (0.1mm)

106= Conversion factor

Results

The results of PCV and RBC analysis of the albino rats treated and control are given below:-

Table 1: PCV counts of albino rats administered aqueous leaf extracts of the experimental plants with their corresponding control.

SNO	Group	PVC (Haematocrit (% Mean ±S.D)
1	Control	40.33 ±4.5
2	Carica Papaya	33.67 ±3.79*
3	Psidium Guajava	43.33 ±3.2
4	M. Indica	38.92 ±5.13

Signifies significant difference at 5% level ($p<0.05$)

Table II: RBC counts of albino rats administered aqueous leaf extracts of the experimental plants with their corresponding control.

SNO	Group	RBC count ($x10^{12}$/L Mean ±SD)
1	Control	4.27 ±1.59
2	Carica Papaya	3.00 ±0.70
3	Psidium Guajava	3.60 ±1.15
4	M. Indica	3.37 ±0.60

Discussion

This current study evaluated the haemolytic effects of the three (3) plants widely being used in our community. The aqueous leaf extracts of Carica Papaya, Psidium Guajava and Mangifera Indica were administered to apparently healthy albino rats with no apparent abnormality resulting in the complication of the blood form in general. The result of PCV in table 1 revealed that, the aqueous leaves extracts of experimental plants were found to exerts some effects on the PCV of the albino rats treated compared with their control (PCV=40.33±4.5) in which Carica Papaya (PCV=33.67±3.79), Psidium Guajava (PCV=43.33±3.22) and Mangifera Indica (PCV=38.92±5.13) . Te extracts resulted in the decrease in PCV value of the treated rats when compared to the normal untreated rats. Statistical analysis of the PCV result using student t-test and Anova analysis shows that Psidium Guajava and Mangifera Indica does not register any significant difference ($p>0.05$) whereas the PCV value of the group administered with aqueous leaf extracts of Carica papaya showed significant difference ($p<0.05$).

However, the results of RBC value presented in table 11 showed a slight decrease in the RBC value of the rats treated with aqueous leaves extracts of Psidium Guajava (RBC=3.60±1.15), Mangifera Indica (RBC=3.37±0.60) and Carica Papaya (RBC=3.0±0.70) compared with that of control (RBC=4.27±1.69) shows no significant difference ($p>0.05$). On the other hand, the analysis of RBC result using Anova analysis of all the groups administered the leaves aqueous extracts shows that the decrease in all the cases was not significant compared to their corresponding control ($p>0.05$). Therefore, considering the PCV result of the group administered with aqueous extract of Carica Papaya result in the decrease of PCV value of the treated rats (PCV=33.67±3.79) compared with their corresponding control (PCV=40.33±4.5) showed significant difference ($p<0.05$) which might be due to red cell haemolysis.

Conclusion

The administration of aqueous leaf extract of Carica Papaya showed significant decrease in PCV value of the experimental rats ($p<0.05$) compared to their corresponding control and hence has haemolytic effect.

Recommendation

The screening of medicinal plants commonly in use in our community should receive greater attention in order to create awareness on the safety or otherwise of the frequently used plants in our society.

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