

Effect of Combined Methanol Extract of *Mucuna Poggei* and *Justicia carnea* Leaves on Hematological Indices and Biochemical Indices of Phenylhydrazine Induced Anaemic Albino Rats

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Abstract

All parts of *Mucuna poggei* and *Justicia carnea* have been known to possess valuable medicinal properties. This calls for the need to determine the effect of combined methanol extract of *mucuna poggei* and *justicia carnea* leaves on hematological and biochemical indices of phenylhydrazine induced anaemic albino rats. The study was done using 60 male albino rats. The rats were divided into five groups of five (5) rats each. Group 1 (non-anaemic) was administered water and feed only which served as Normal control. Group 2 was administered Phenylhydrazine only which served as negative control. Group 3 through 5 were Phenylhydrazine induced anaemia treated with combined extract (1:1) at 200 mg/kg, 400 mg/kg and 600 mg/kg respectively. The results showed a significant ($p < 0.05$) increase in Hepatic and renal biomarkers (ALT, AST, ALP, Urea, Creatinine) of the anaemic (negative) control. The combined extract significantly restored the alteration through significant ($p < 0.05$) dose dependent decrease in the assayed hepatic and renal function biomarkers. The hematological indices of the treated rats were maintained compared to the anaemic control. The study showed great potential of combined methanol extract of *Mucuna poggei* and *Justicia carnea* leaves capable of ameliorating anaemia.

Keywords: Anaemia, combined extract, hematology, biochemical indices, *mucuna poggei*, *Justicia carnea*.

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INTRODUCTION

Health is the subject of priority as far as life is concerned but despite efforts to maintain good health, man still confronts disease conditions which are due to exposure to chemical agents in the environment (Kumar *et al.*, 2009). Though the body system is made in such a way that it tackles invading foreign substances, in most cases the body system needs to be protected, enhanced and activated (Effiong *et al.*, 2013). Anemia is a serious global public health problem associated with an increased risk of morbidity and mortality especially in developing countries in Africa such as Nigeria. In poorer malaria-endemic countries, anemia is one of the commonest preventable causes of death in children under 5 years and in pregnant women and thereby poses a great threat to global healthcare (Ashour, 2014). This disease is characterized by the decrease of the hemoglobin rate to less than 13 g/dl in males or 12 g/dl in females (Onyeabo *et al.*, 2017). This ability to activate the body defense mechanism or to protect the body

system against anaemia has been found reported using medicinal plants. For ages, plants have been a good source of food and they provide essential nutritional values, medicinal properties and notable physiological effect to life (Emelike and Dapper, 2013).

It has been observed that there is an increased awareness on the importance of wild or semi-wild plants owing to their high vitamin and mineral contents. Most of these are utilized to provide not only nutrients, but also traditional treatment for various ailments (Hassan and Ngaski, 2007). Such plants are known legumes like *Mucuna poggei* and *Justicia carnea*, their contents are scientifically proven to be effective. It is very important to note their numerous applications such as antioxidant, antibacterial, anti-allergic, analgesic, anti-tumoral activities and many others. These plants have been useful in treating clinical conditions in which the number of circulatory red blood cells (RBCs) is insufficient to meet physiological needs (Reid *et al.*, 2008). *Mucuna poggei*

and *Justicia carnea* are well known plants in tropical areas for treatment of many health issues such as cardiovascular diseases, tonic for blood replacement, as stimulant and many others. It has been reported that different parts of these plants contain phyto-constituents with proven biological activities. However, due to the increasing consumption of especially *Justicia carnea*, it is important to study its vitamin and mineral contents with the notion that the intake of natural plants is being associated with reduced risk of diseases (Ashour, 2014) and this had given rise for a study designed to determine the effect of combined methanol extract of *Mucuna poggei* and *Justicia carnea* leaves on hematology, liver and kidney function indices of Phenylhydrazine induced anaemic albino rats.

MATERIALS AND METHODS

Plant Collection and Identification

Mature leaves of *Mucuna poggei* and *Justicia carnea* were collected from Enyigugu, Aboh Mbaise L.G.A Owerri, in Imo state, and identified by Dr. C Duru of the department of Environmental Biology, Federal Polytechnic Nekede, Owerri, Imo state.

Preparation of the Methanol Plant Extract

The leaves of *Mucuna poggei* and *Justicia carnea* were washed and air dried at room temperature. Then it was pulverized and macerated in 2L of methanol for 72 hrs. The mixture was decanted through a glass funnel fitted with Whatmann No. 1 filter paper. The filtrate was then allowed to evaporate to dryness using oven at 45°C. The dried extract was afterwards stored until when required.

Experimental Animals

Twenty five albino rats weighing between 70g to 110g were purchased from the animal house unit of University of Nigeria Nsukka, Enugu State. The animals were housed in locally fabricated cages and were allowed to a seven days acclimatization period before the commencement of the study. The experimental animals were maintained on animal feed and given water *ad libitum*.

Evaluation of Acute Toxicity of the combined Extract

The acute toxicity of the combined extract was determined by evaluating the median lethal dose (LD50) using 18 mice by following the method of Lorke (1983).

Induction of Anemia

Anemia was induced intraperitoneally in the rats except normal control using 20mg/kg b.w. of

phenylhydrazine for five consecutive days in order to induce anemic.

Experimental design

The rats were divided into five groups of five (5) rats each as follows:

Group 1: (non-anemic) was administered water and feed only which served as Normal control.

Group 2: was administered Phenylhydrazine only which served as negative control

Group 3: Phenylhydrazine induced anaemia treated with combined extract (1:1) at 200 mg/kg

Group 4: Phenylhydrazine induced anaemia treated with combined extract (1:1) at 400 mg/kg

Group 5: Phenylhydrazine induced anaemia treated with combined extract (1:1) at 600 mg/kg

After 14 days treatment, the rats were sacrificed by means of cervical dislocation and the sera collected through cardiac puncture and used for analysis.

Hematological indices

The determination of erythrocyte count was done by haemocytometry following the method of Ochei and Kolhatkar (2008). The packed cell volume was determined using the microhaematocrit centrifuge (Jouan A13 model). The haemoglobin (Hb) concentration was measured spectrophotometrically by cyanomethaemoglobin method. The platelet count was obtained using the haemocytometer. Estimation of MCV, MCH and MCHC were done by calculation using standard formula

Biochemical analysis

All parameters were determined using standard test kits and analytical grade reagents and chemicals. Serum alanine amino transferase (ALT) and aspartate aminotransferase (AST) activities were estimated by the method of Reitman and Frankel (1957). The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss (1976). Urea level was determined using Berthel's urease reaction method, as described by Fawcett (1960). Serum creatinine was determined using direct end point according to Henry (1974) as described in redox commercial kit.

STATISTICAL ANALYSIS

The results were presented as mean \pm SD. Total variations present in a set of data were estimated by One Way Analysis of Variance (ANOVA) using Duncan post hoc test and *p* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

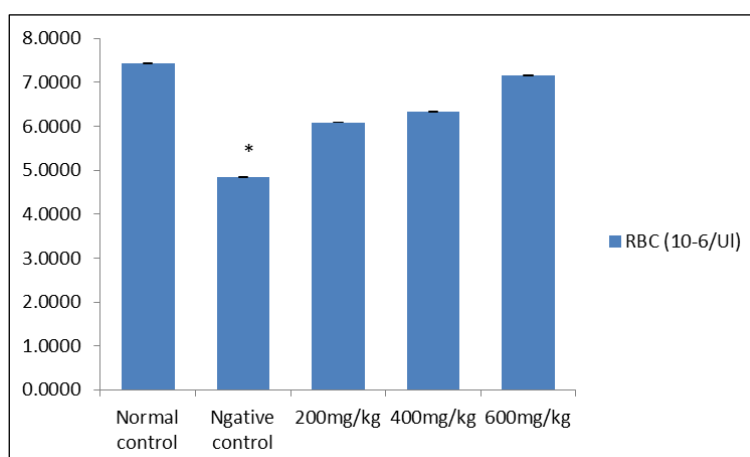


Figure 1: Effect of combined methanol extract of *Mucuna poggiei* and *Justicia carnea* leaves on RED blood cell (RBC) concentration of Phenylhydrazine induced anaemic albino rats

The result of the effect of the combined extract on RBC (Fig 1) showed dose dependent significant ($p < 0.05$) increase in RBC of the extract treated groups

compared to the negative control. There is non-significant ($p > 0.05$) difference between the normal control and the 600mg/kg extract treated group.

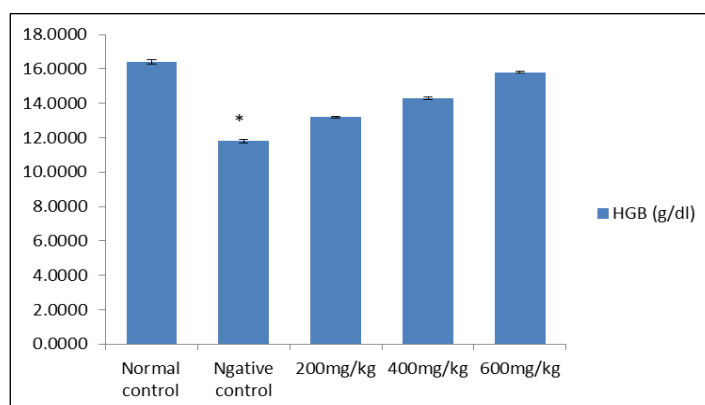


Figure 2: Effect of combined methanol extract of *Mucuna poggiei* and *Justicia carnea* leaves on hemoglobin (HGB) concentration of Phenylhydrazine induced anaemic albino rats

The HGB result (Fig 2) showed dose dependent significant ($p < 0.05$) increase in HGB level of the extract treated groups compared to the negative control. There

was significant ($p < 0.05$) decrease in the HGB of the negative control compared to the normal control.

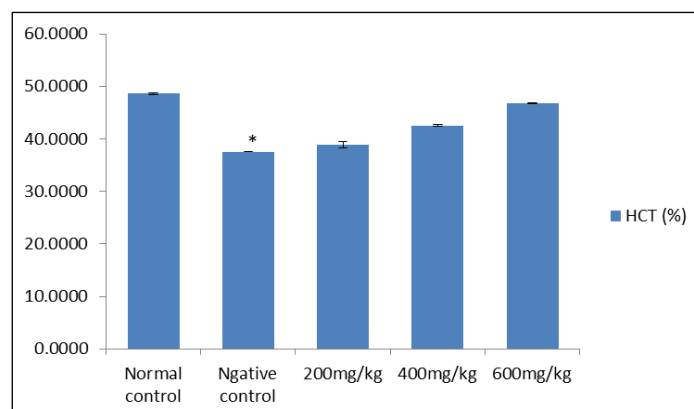


Figure 3: Effect of combined methanol extract of *Mucuna poggiei* and *Justicia carnea* leaves on hematocrit (HCT) concentration of Phenylhydrazine induced anaemic albino rats

The result of the hematocrit concentration (Fig 3) showed dose dependent significant ($p < 0.05$) increase

in HCT level of the extract treated groups compared to the negative control.

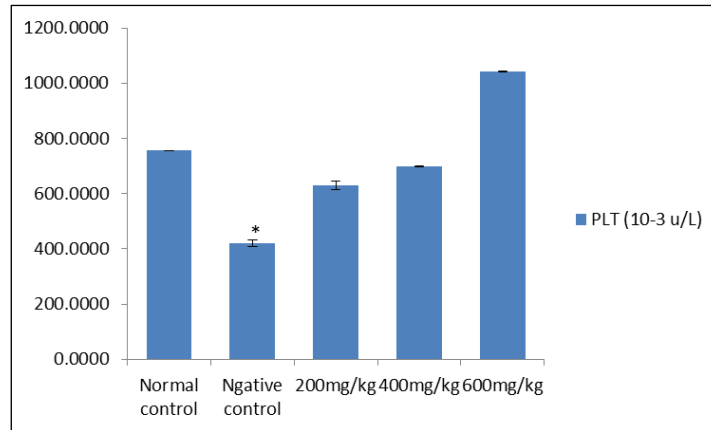


Figure 4: Effect of combined methanol extract of *Mucuna pogeii* and *Justicia carnea* leaves on platelets (PLT) concentration of Phenylhydrazine induced anaemic albino rats

The result (Fig 4) showed dose dependent significant ($p < 0.05$) increase in PLT level of the extract treated groups compared to the negative control.

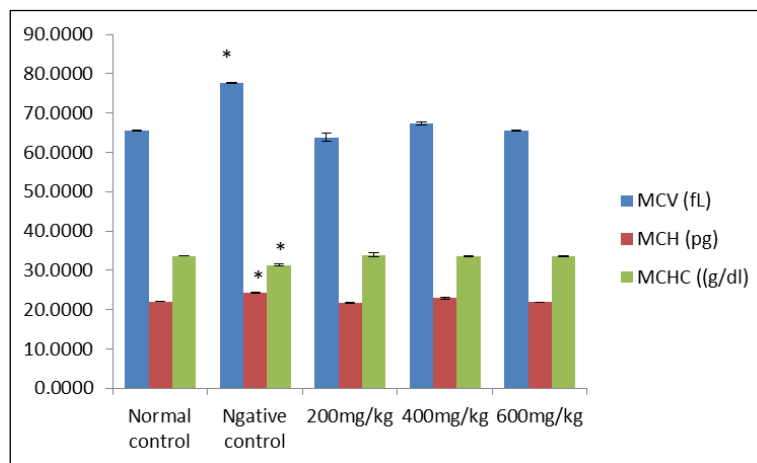


Figure 5: Effect of combined methanol extract of *Mucuna pogeii* and *Justicia carnea* leaves on MCV, MCH and MCHC levels of Phenylhydrazine induced anaemic albino rats

The result (Fig 5) showed dose dependent significant ($p < 0.05$) decrease in MCV, MCH and MCHC levels of the extract treated groups compared to the

negative control. There was also non-significant ($p < 0.05$) difference among the extract treated groups.

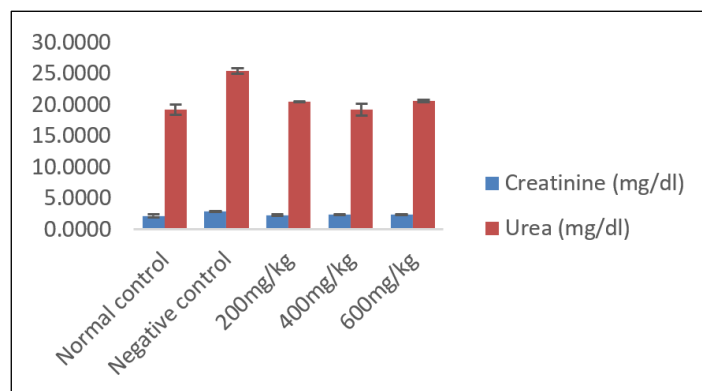


Figure 6: Effect of combined methanol extract of *Mucuna pogeii* and *Justicia carnea* leaves on creatinine and urea levels of Phenylhydrazine induced anaemic albino rats

There was non-noticeable significant ($p < 0.05$) difference in the creatinine level of all the groups but there was a slight significant ($p < 0.05$) dose dependent

decrease in the urea as against that of the anemic control (Fig 6).

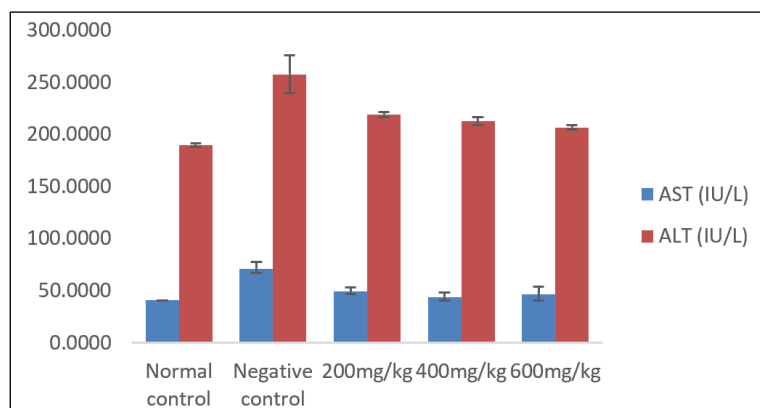


Figure 7: Effect of combined methanol extract of *Mucuna poggii* and *Justicia carnea* leaves on AST and ALT activities of Phenylhydrazine induced anaemic albino rats

The results (Fig 7) showed significant ($p < 0.05$) decrease in AST and ALT of the extract treated groups compared to the negative control. There is non-

significant ($p > 0.05$) difference between the normal control, 400mg/kg and 600mg/kg extract treated groups.

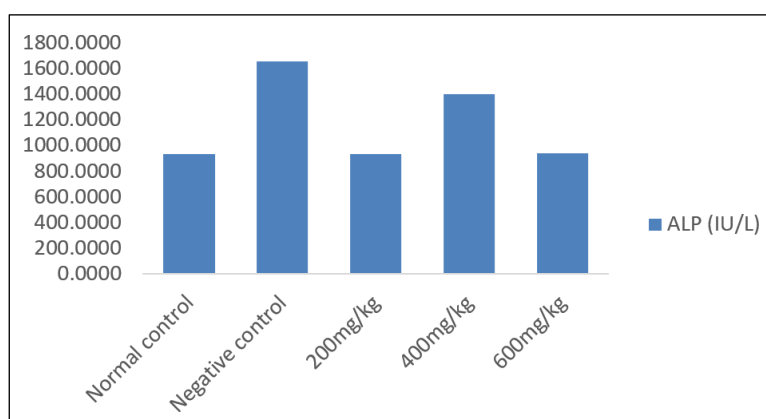


Figure 8: Effect of combined methanol extract of *Mucuna poggii* and *Justicia carnea* leaves on ALP activity of Phenylhydrazine induced anaemic albino rats

The above result showed significant ($p < 0.05$) decrease in ALP of the extract treated groups compared to the negative control. There is non-significant ($p > 0.05$) difference between the normal control, 200mg/kg and 600mg/kg extract treated groups.

DISCUSSION

Anaemia is among the common blood associated ailments which affect people all around the globe (Priya *et al.*, 2017). It can emerge at all phases of the life process, but it occurs mostly in pregnant women and young children (Shende *et al.*, 2017). The occurrence of anaemia is higher in the tropics due to the high prevalence of malaria and other parasitic infections that could lead to decrease in circulating red blood cells or hemoglobin level (Toma *et al.*, 2015). The present study evaluated the effect of combined methanol extract of *Mucuna poggii* and *Justicia carnea* leaves on hematological, liver and kidney function indices in

albino rats. In the acute toxicity assay, there was no mortality even at the highest administered dose of 5000mg/kg which implies that the combined extract is safe. The administration of PHZ in rats produces a decreased PCV level which agreed with previous reports by Berger (2007), that reported reduction in PCV in hemolytic anaemia induced by 2, 4-DPHZ caused that leads to oxidation of HB and sulfhydryl groups of the erythrocytes membrane and enzymes leading to haemolysis of erythrocytes (Nadro and Modibbo, 2014). However, Oral administration of combined methanol extract of *Mucuna poggii* and *Justicia carnea* leaves produces a significant dose-dependent increase in PCV, RBC, HB and a significant decrease in WBC, MCV, MCH after 14 days treatment.

This agreed with earlier findings by Nadro and Modibbo, (2014) where oral administration of 250 and 500mg/kg body weight aqueous extract of *P. erinaceus*

Stem Bark significantly increased PCV, Hb, RBC and significantly decreased MCH and MCHC in PHZ induced anaemic rats. Similarly, treatment of 2,4-DPHZ induced anemic rabbits with *Hibiscus sabdariffa* anthocyanin extract produces a significant ($P < 0.05$) increase in, RBC counts, PCV and Hb and a decrease in WBC counts (Ologundudu *et al.*, 2009). This positive effect seen in this study could probably be due to the ability of combined methanol extract of *Mucuna poggie* and *Justicia carnea* leaves to protect the RBC against oxidative haemolysis induced by 2,4-DNPH. There is a significant dose dependent increase in Platelet level of the extract treated groups compared to the negative control. This implies that the combined extract could also prevent excessive bleeding and blood lost. Liver function parameters used to detect the presence of liver disease or potential harm to the liver include the serum level of the enzymes AST, ALT and Alkaline phosphatase.

Usually, any kind of liver injury can cause a rise in ALT, and the release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage (Chikwendu *et al.*, 2015). The results of this study showed that there was a significant ($p < 0.05$) increase in the levels of ALP, AST, and ALT in the negative control group compared to the values of the normal control group. But the groups treated with different doses of combined methanol extract of *Mucuna poggie* and *Justicia carnea* leaves were observed to have a very significant ($p < 0.05$) reduction in the levels of these enzymes bringing their values closer to that of the normal control. Higher levels of blood urea nitrogen and creatinine could be a sign of an underlying condition affecting the kidneys (Raj, 2014). However, the urea and creatinine (mg/ dl) was assayed for indication nephrotoxicity. There was non-noticeable significant ($p < 0.05$) difference in the creatinine level of all the groups but there was a slight significant ($p < 0.05$) dose dependent decrease in the urea as against that of the anemic control.

CONCLUSION

The results of the present study revealed a significant anti-anemic property of combined methanol extract of *Mucuna poggie* and *Justicia carnea* leaves in phenylhydrazine-induced anaemia in experimental rats. The results also suggest that the combined extract may promote liver function parameters, maintain normal kidney function indices. The anti-anaemic activity of the plant could be due to the presence of important bioactive compounds which are known to possess erythrocytes protective abilities hence, reversing the anaemia induced in the rats.

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