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Advances in Pharmacology and Clinical Trials
ISSN: 2474-9214

Effect of Methanol Extract of the Ripe Fruit Peels of *Musa Paradisiaca* on Some Haematological and Biochemical Indices in Male Wistar Rats

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Research Article

Volume 4 Issue 1

Received Date: March 01, 2019

Published Date: March 25, 2019

DOI: 10.23880/apct-16000154

Abstract

The present study evaluated the effect of methanol extract of the fruit peels of ripe plantain (an agricultural waste) on some haematological and biochemical indices in male Wistar rats. Phytochemical screening of the ripe peel extract was carried out using standard methods. The oral median lethal dose (LD_{50}) was determined. Twenty-four (24) male Wistar rats were randomly assigned into 4 groups of 6 rats per group. Group 1 served as control. Groups 2 - 4 were administered 285.00, 570.00 and 855.00 mg/kg per body weight respectively of the ripe plantain peel extract for a period of 14 days. Blood samples were collected by cardiac puncture and analyzed for some hematological and biochemical parameters. Phytochemical screening of the ripe peel extract indicated the presence of flavonoids, tannins and cardiac glycosides. Acute toxicity study gave an LD_{50} of 2849.56 mg/kg. Treatment of experimental animals with the extract caused a dose dependent increase in white blood cell count (WBC), red blood count (RBC), packed cell volume (PCV) and hemoglobin concentration (Hb) in the medium and high dose groups. Total cholesterol, triglycerides, very low-density lipoprotein-cholesterol, low-density lipoprotein cholesterol and highdensity lipoprotein-cholesterol did not show any significant changes ($p>0.05$) between control and treatment groups. Also, there were no significant changes ($p>0.05$) in the serum activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. These findings suggest that

the ripe fruit peels of *M. paradisiaca* are non-toxic and could positively impact hematopoiesis. Hence, these peels could be further exploited for nutritional and pharmacological purposes.

Keywords: Plantain Peels; Phytochemical Screening; Acute Toxicity; Hematology; Liver Enzymes; Lipid Profile

Abbreviations: WBC: White Blood Cell Count; RBC: Red Blood Count; PCV: Packed Cell Volume; HB: Hemoglobin Concentration; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; RBC: Red Blood Cell Count; PLT: Platelet Count; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin Concentration.

Introduction

Musa paradisiaca Linn (Plantain) is a monoherbaceous plant belonging to the Musaceae family [1]. The origin of plantain has been traced to the southern part of India from where it has spread to the tropical and sub-tropical zones of Africa, Asia, central and southern America [2]. The mature plant can attain a height of up to 9 m [3]. The plant portion above the ground is a false stem (pseudostem) consisting of concentrically formed leaves from the centre of which develops the inflorescent stalk. The rhizome or true stem of this plant is present underground. Near the tip of the flower stalk are groups of sterile male flowers subtended by brilliant purple bracts. A cluster of female flowers which give rise to the fruits are found in the lower part of the flower stalk [4]. The fruit are oblong, 5 - 7 cm long in the wild plant and longer in the cultivated varieties [3]. The unripe fruits are green in colour but on ripening the colour changes to golden yellow [5]. The fruits can be consumed when ripe or unripe. It contributes substantially to the nutrition of local populations [6]. Aside from being utilized as food, the plantain fruit alongside other parts of the plant are valuable in ethno medicine for the treatment of various ailments [4]. During the processing of the plantain fruits for human consumption, the outer peels (epicarp) are usually discarded as waste or utilized as fodder for domestic animals such as goats and sheep [7]. In the cities, the plantain fruit peels could contribute to the solid waste load and environmental pollution [8].

In an effort to convert the plantain fruit peels into useful products, they have been subjected to various investigations. Nutritionally, the peels have been found to be rich in some macro and micro nutrients [9-11]. Phytochemical analysis has indicated the presence of bioactive compounds such as flavonoids, alkaloids, tannins [9,10]. Pharmacological activities attributed to the plantain peels include antimicrobial [10,12]; antioxidant [10,13,14] and wound healing [13]. The effect of the plantain peels on growth performance of rabbits was reported by Ogunsipe and Agbede [7]. Data has also been presented on the effects of plantain peel meal on organ weight and blood parameters in broiler chicken [15]. An evaluation of the effect of ripe plantain peel-based diet on some enzymes of the liver, heart and kidney of albino rats has been carried out [16]. There is insufficient information in the literature concerning toxicity of the plantain fruit peels. Hence, this study was carried out to assess the effects of methanol extract of ripe plantain peels on some haematological and biochemical indices in male Wistar rats. It is expected that the outcome of this study will provide additional information on the safety of the ripe plantain fruit peels upon exposure to animals.

Materials and Methods

Sample Collection and Preparation of Extract

High quality ripe fruits of plantain were purchased from a local market in Itu, Akwa Ibom State, Nigeria. All the fruits were at stage 6 of the ripening scale described by Forster, et al. [17]. They were authenticated at the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria. The fruits were washed with potable water to remove any dirt and drained. The apex of the fruits and stalk were cut off and discarded using a sharp stainless steel knife. The fruit peels were carefully separated from the pulps, air dried for a period of two weeks, chopped into small pieces and pulverized in a milling machine. The crude extract was prepared by macerating 50 g of the pulverized sample in 500 ml of methanol for 72 hours, with intermittent shaking of the mixture. Subsequently, the mixture was filtered and the filtrate was concentrated in a rotary evaporator at 40^{o}C. The resulting crude extract was preserved in a refrigerator at -4°C.

Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade.

Phytochemical Analysis

Phytochemical screening was carried out using standard procedures [18,19].

Determination of LD_{50}

This was determined by the method of Lorke [20] using Swiss albino mice (n=12). The study was conducted in two phases after the mice had been fasted overnight. In the first phase, nine mice were divided into three groups of three animals per group. They were orally administered 1000, 2000 and 3000 mg/kg bw of the extract respectively. The number of deaths in each group within 24 hours was noted. In the second phase which was deduced from the first phase, three mice were grouped into three groups of one mice per group and treated with doses of 2600, 2800 and 2900 mg/kg bw of the extract respectively. They were also observed for 24 hours. The LD_{50} was calculated using the formula $LD_{50}=\sqrt{ab}$, where 'a' is the maximum dose producing 0% mortality and 'b', the minimum dose producing 100% mortality.

Experimental Design

Twenty-four male albino rats of the Wistar strain weighing 160-200 g were used in this study. The animals were obtained from the Animal House Facility of Department of Pharmacology and Toxicology, University of Uyo, Nigeria. The animals were fed standard laboratory diet, housed in wire-meshed cages, maintained under standard environmental conditions of temperature, 23 ± 2°C, relative humidity 60% and a 12hour light/dark cycles. They were allowed an acclimatization period of seven days before commencement of the experiment. The rats were randomly selected into four groups of six animals each. Group I served as the control and received distilled water. Groups II, III and IV were administered 285.00 mg/kg, 570.00 mg/kg and 855.00 mg/kg body weight respectively of the ripe plantain peel extract representing low, medium and high dosages. Extract administration was carried out by oral gavage once daily between the hours of 8-10 am before administration of feed for a period of 14 days. Institutional approval was obtained from the Postgraduate School, University of Uyo, Nigeria. The animal studies were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" [21]. At the end of the experimental period (14 days), the animals were anaesthetized using chloroform fumes after an overnight fast. Blood samples were collected via cardiac puncture. One portion of the blood sample was collected into sterile EDTA (anti-coagulant) bottles and used for whole hematological analysis. Blood samples were also collected into sterile plain test tubes for the preparation of serum. The serum samples were obtained from the coagulated blood after centrifugation at 3000 rpm for ten minutes using a bench top centrifuge (MSE Minor, England). The sera were stored frozen at -20^{0}C until required for analysis of biochemical parameters.

Determination of Haematological Parameters

Haematological parameters were determined within two hours of sample collection using an automated haematology analyzer (Sysmex KX-21N, Japan) at the University of Uyo Teaching Hospital, Uyo, Nigeria. The haematological indices determined include Haemoglobin concentration [Hb], Packed cell volume (PCV), White blood cell count (WBC), Red blood cell count (RBC), Platelet count (PLT), Mean cell haemoglobin (MCH), Mean cell volume and Mean cell haemoglobin concentration (MCHC).

Biochemical Analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined using the Randox kit (Randox Laboratories, England), according to manufacturer's instructions. Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were also estimated using the diagnostic reagent kit supplied by Randox Laboratories, England according to the manufacturer's instructions.

Data Analysis

Data obtained were expressed as Mean ± SEM and analysed using the one-way ANOVA. The difference between mean values were compared using the Least Significant Difference (LSD). Values of $P<0.05$ were considered to be statistically significant [22].

Results

Phytochemical Screening

The phytochemical constituents of the methanol extract of the ripe plantain peels indicated the presence of tannins, flavonoids and cardiac glycosides (Table 1).

Constituent
Alkaloid -
Saponin -
Tannin + +
Flavonoids + +
Phlobatannins -
Cardiac glycosides + +

Table 1: Phytochemical composition of the methanol extract of ripe plantain peels.
Key: + present; ++ moderately present; - absent.

Acute Toxicity

Table 2 shows the result of acute toxicity test. In stage one, mortality was observed only in the animals treated with 3000 mg/kg body weight of the extract. While in stage two, there was mortality in the group that received 2900 mg/kg body weight of the methanol extract of the ripe peels of plantain. Thus, the oral median lethal dose (LD_{50}) of the extract in mice was calculated to be 2849.56 mg/kg body weight.

Stage	Dose (mg/kg body weight)	Fraction of death	%Mortality
1	1000	0/3	0
	2000	0/3	0
	3000	3/3	100
2	2600	0/1	0
	2800	0/1	0
	2900	1/1	100

Table 2: Result of acute toxicity test of the methanol extract of ripe plantain peels.

Effect of Methanol Extract of the Ripe Peels of Plantain on Haematological Indices

The effect of methanol extract of ripe peels of plantain on haematological indices in male Wistar rats is shown in Table 3. Haemoglobin concentration, PCV, WBC values and total RBC count showed significant ($p<0.05$) dose dependent increases across the treatment groups compared to the control group. Total Platelet count, MCV, MCH and MCHC did not show any significant differences between the treatment groups.

Group	[Hb] (g/dl)	PCV (%)	WBC(x10^{12}/L)	RBC(x10^{12}/L)	PLT(x10^{9}/L)	MCV (fl)	MCH (pg)	MCHC(g/dl)
1(control)	12.50+1.80	35.00+0.40	10.60±1.2	4.50+0.20	750.00+29.10	53.00+3.20	16.00+0.40	31.00+1.10
2(285.00mg/kg) (Low dose)	12.80+2.10	38.00+1.00	13.00+2.30	5.40+0.30	768.00+29.30	50.00+2.20	18.00+0.20	30.00+0.40
3(570.00mg/kg) (Middle dose)	13.80+2.40^{*a}	40.00+0.40^{*a}	15.00+1.20*	6.70+0.30*	770.00+18.60	54.00±3.30	17.20±1.7	31.00+0.40
4(855.00mg/kg) (High dose)	14.45+2.10^{*ab}	42.00+0.10^{*ab}	18.60+1.60^{*ab}	8.40+0.40^{*ab}	780.00+32.40	48.00±1.8	19.00+0.40	30.80±0.50

Table 3: Effect of methanol extract of the ripe peels of plantain on haematological indices in male Wistar rats.
Values are expressed in Mean + SEM (n=6);* significantly different ($p<0.05$) compared with control group; [a]significantly different ($p<0.05$) compared with group 2; [b]significantly different ($p<0.05$) compared with group 3

Effect of Methanol Extract of the Ripe Peels of Plantain on Serum Lipid Profile

There were no significant differences in the values obtained for total cholesterol (TC), triglyceride (TG), HDL, LDL and VLDL-C levels of experimental animals (Table 4).

Groups	TC (mmol/L)	TG (mmol/L)	VLDL (mmol/L)	HDL (mmol/L)	LDL (mmolL)
1(control	2.30± 0.10	0.86± 1.10	0.39±0.08	1.40±1.20	0.51±0.10
2(285.00mg/kg) (Low dose)	2.50±1.10	0.82±0.40	0.37±0.11	1.70±1.10	0.43±0.12
3(570.00mg/kg) (middle dose)	2.40± 0.00	0.90± 0.10	0.41±0.07	1.80±0.10	0.19±0.05
4(855.00mg/kg) (high dose)	2.50± 0.00	0.84±0.10	0.38±0.09	1.80±0.60	0.32±0.09

Table 4: Effect of the methanol extract of ripe peels of plantain on lipid profile of male Wistar rats.
Values are expressed in Mean + SEM (n=6); p>0.05 in all treatment groups

Effect of methanol extract of the ripe peels of plantain on serum enzymes

Serum enzymes (AST, ALT and ALP) did not exhibit any significant differences between the test groups (Table 5).

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
1(control)	45.00 + 1.00	19.00+ 0.10	101.00 + 0.10
2(298.8mg/kg) (Low dose)	44.00 + 0.10	20.00 + 0.50	102.00 + 0.40
3(579.6mg/kg) (middle dose)	47.00 + 0.30	22.00 + 0.00	100.00 + 0.50
4(869.9mg/kg) (High dose)	45.00 + 0.30	20.00 + 0.00	102.00 + 0.00

Table 5: Effect of the methanol extract of ripe peels of plantain on serum enzymes in male Wistar rats.
Values are expressed in Mean + SEM (n=6); p>0.05 in all treatment groups.

Discussion

Phytochemical screening of the methanol extract of the ripe peels of *M. paradisiaca* indicated the presence of tannins, flavonoids and cardiac glycosides. The phytochemicals possess a wide range of biological activities and are largely responsible for the pharmacological properties of plants [23]. Cardiac glycosides have stimulatory effect on the heart muscle and could be used in the treatment of cardiac failure [24]. The health benefits of flavonoids have been attributed to their antioxidant properties [25]. Tannins possess antibacterial, anti-inflammatory, antiviral, astringent and wound healing potentials [26]. The presence of these bioactive compounds in the ripe peels of plantain is an indication of their potential medicinal applications. There are reports on the antimicrobial, antioxidant and wound healing properties of plantain peel extract [12,13].

The evaluation of acute toxicity is an early step in the toxicological investigation of test substances [27]. In the present study, the oral minimum lethal dose (LD_{50}) of the methanol extract of the ripe peels of *M. paradisiaca* was estimated to be 2849.56 mg/kg body weight. Hence, the extract can be described as none toxic with reference to the scale proposed by Lorke [20].

The aminotransferases (AST, ALT) are intracellular enzymes while ALP is a membrane bound enzyme [28,29]. When there is damage to the hepatocytes, these enzymes leak into circulation and their activities in serum increase significantly [30]. In the present study, administration of the methanol extract of ripe plantain peels did not induce any significant alterations in the activities of these enzymes (p>0.05). This is in agreement with other studies which indicated that the inclusion of pulverized peels of ripe plantain into the diet did not alter activities of serum diagnostic enzymes [16]. It can therefore be inferred that the methanol extract of the ripe peels of plant does not inflict hepatocellular injury on the experimental animals.

The assessment of haematological parameters is useful in the determination of haematoxic potential of a test substance [31]. The present study has demonstrated that the extract of ripe plantain peels induced a dose dependent increase in WBC count. The observed increase in this parameter could be regarded as a normal immune response to invasion by a foreign entity [32]. Oyedeji, et al. [15] had reported significant increase in WBC of broiler chicken maintained on plantain fruit peel-based diet. In the present study, there was a dose dependent increase in [Hb], PVC and RBC. This is an indication that the extract could stimulate haematopoietic activity. The haematopoietic potential of plant extracts has been attributed to bioactive components such as tannins and flavonoids [33]. It has been further observed that flavonoids exert multiple beneficial effects on human health due to their antioxidant and free radical scavenging activities coupled with anti-inflammatory, anti-viral and anti-cancer properties [34,35]. The result of this study

corroborates the report of Oyedeji, et al. [15] who recorded significant improvements in these indices when broiler chicken was fed ripe plantain fruit peel supplemented diet. This study has also shown that there were no significant changes in platelet count, MCV, MCH and MCHC. Failure of the extract to impact platelet count suggests that the extract had no effect on the synthesis of thrombopoietin [36]. MCH and MCHC are indices that define the concentration of haemoglobin within the erythrocytes [37]. The results obtained for MCH and MCHC indicate that the haemoglobin concentration and oxygen carrying capacity of the erythrocytes were not compromised [32]. MCV is an expression of the average volume of a single red blood cell [37]. The extract did not impact this parameter.

Lipid profile is an important indicator of cardiovascular health [38]. The parameters of interest here include serum concentrations of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C). An elevation in the serum concentrations of TC, TG and LDL-C with low levels of HDL-C is a risk factor for the development of atherosclerosis [38,39]. The present study has demonstrated that the administration of methanol extract of the ripe fruit peels of plantain does not induce any significant alterations in the lipid profile. Hence, the peels would not compromise cardiovascular health.

Conclusion

This study has shown that the ripe peels of *M. paradisiaca* contain bioactive compounds such as tannins, flavonoids and cardiac glycosides. The value obtained for LD_{50} indicates that the extract is non-toxic and could be nutritionally safe if ingested. The extract exerts positive modulation of hematopoietic activities as evidenced by its ability to increase [Hb], PCV, RBC. The study observes the need for the pharmacological and nutritional potentials of the ripe fruit peels of *Musa paradisiaca* to be further explored.

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