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Haematological and Biochemical effects of a combination of leaves of *Telfairia occidentalis* and *Mucuna pruriens* in male rats

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Abstract

Medicinal plants are useful in the treatment and management of certain ailments due to their affordability and minimal or no side effects compared to conventional drugs. The aim of this research was to investigate the effect of administration of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaf extracts on haematological and biochemical parameters in male wistar albino rats. The phytochemical analysis was carried out using standard analytical methods. The fourteen-day acute toxicity study was done using Lorke's method.

The haematological and biochemical analysis were carried out using standard diagnostic techniques. The phytochemical analysis revealed the presence of important phytochemicals. The result of the fourteen-days acute toxicity study revealed that the LD_{50} for the aqueous, ethanol, methanol and n-hexane extract is 3808mg/kg bw. The effect of the twenty-eight days treatment with the extracts at 200mg/kg bw on the haematological analysis showed that aqueous, ethanol and methanol extracts caused a significant ($p<0.05$) increase in the packed cell volume, haemoglobin, red blood cells and platelets compared with the experimental control. The aqueous, ethanol and methanol extracts did not alter the biochemical parameters of the animals. The n-hexane extract caused a significant ($p<0.05$) increase in the white blood cells count, aspartate transaminase, alkaline phosphatase, bilirubin concentrations, urea, creatinine and bicarbonate concentrations of the treated rats compared with the experimental control. The results obtained suggest that the aqueous, ethanol and methanol leaf extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* can increase blood concentration without upsetting the biochemical parameters in experimental rats.

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Introduction

Medicinal plants have been of great use since time immemorial in the prevention, management and treatment of certain ailments. Medicinal plants are widely used in the developing countries mainly because they are readily available and cheaper than conventional drugs. Plants contain useful phytochemicals which may be responsible for its pharmacological activity. The phytochemical content and pharmacological actions of many plants still remain unassessed by scientific research to define their efficacy and safety (Ahn, 2017). Although most of these medicinal plants are in great use and recommendation by the traditional herbal users, there is no scientific backing on their effect on most of the haematological and biochemical parameters. The high cost of conventional drugs used in the treatment of blood shortage as well as its attendant side effects and contraindications have continued to impede the successful treatment of anaemia. This has triggered the recourse to complimentary alternative medicine without investigating their effect on essential biochemical parameters. Anaemia is the commonest red cell disorder associated with several conditions such as nutritional deficiencies, genetic defects, blood loss, as well as drug toxicity, affecting people of all ages although the people at greater risk are the elderly, young women of child bearing age and infants (Adebayo *et al*., 2017).

In the rural areas, many people depend on leaves of *Telfairia occidentalis* for the treatment of anaemia and as blood booster (Nwauzoma and Dappa, 2013; Verma and Baksh, 2013). *Telfairia occidentalis* is widely cultivated in Africa and beyond for its palatable and nutritious leaves. The leaves have comparatively high nutritional value than other tropical vegetables (Olorunfemi *et al*., 2014). The pharmacological properties of *Telfairia occidentalis* which include haematinic properties of the leaf extract was reported (Dina *et al*., 2006), improved spermatogenesis (Nwangwu *et al*., 2007), hepatoprotective properties (Oboh, 2005), safe antidote for cyanide poisoning (Bolaji and Olabode, 2011).

Mucuna pruriens is a tropical twining herb commonly known as Velvet bean belongs to the family Fabeaceae (Vermal *et al*., 2014). The plant is famous for the extreme itchiness it produces on contact, particularly with the young foliage and the seed pods due to the presence of protein, mucunain and serotonin [5- hydroxytryptamine (5-HT)] (Aray *et al*., 1953; Agharkar, 1991). Some of the pharmacological importance of *Mucuna pruriens* that have been studied include aphrodisiac activity (Sekar *et al*., 2009), hypoglycemic activity (Rathi *et al*., 2002), antivenom activity (Fung *et al*., 2009; Natarajan *et al*., 2012), antioxidant activity (Rajeshwar *et al*., 2005) and antitumor activity (Rajeshwar *et al*., 2005; Natarajan *et al*., 2012).

The leaf extracts of *Telfairia occidentalis* and *Mucuna pruriens* have been used separately in the treatment of some diseases. The effect of *Mucuna pruriens* seed on some haematological parameters and biochemical parameters has earlier been investigated in rats (Adepoju and Odubena, 2009). However, there is no documentation on the effect of these two extracts given as a combination on the haematological and biochemical parameters when given as combination.

Synergism is the interaction of a drug with another to produce increased activity, which is greater than the sum of the effects of the two drugs given separately. A synergistic effect can be beneficial or harmful. The aim of this study is to investigate the combined effect of the administration of the leaf extracts of *Telfairia occidentalis* and *Mucuna pruriens* on some haematological and biochemical parameters of male wistar rats. If the synergistic effect of *Telfairia occidentalis* and *Mucuna pruriens* is proven to be efficacious in the treatment of anaemia without upstaging essential biochemical parameters it will be a worldwide relief.

Materials and methods

Plant collection

The leaves of *Telfairia occidentalis* and *Mucuna pruriens* were collected from Nnamdi Azikiwe

University, Awka. Anambra State. The samples were validated by a botanist in the Department of Botany, Nnamdi Azikiwe University, Awka. The Voucher number for *Telfairia occidentalis* and *Mucuna pruriens* as deposited in the Nnamdi Azikiwe University herbarium was 8 and 10 respectively.

Plant preparation

The leaves of *Telfairia occidentalis* and *Mucuna pruriens* were properly washed separately and air dried at room temperature for two weeks. The dried leaves were ground into powder using corona manual grinding machine. Exactly 200g each of the ground leaves of a combination of *Telfairia occidentalis* and *Mucuna pruriens* (1:1) were soaked in 1 litre of distilled water, 80% ethanol, 80% methanol and n-hexane for 24 hrs to yield aqueous, ethanol, methanol and n-hexane extract respectively. It was sieved and filtered using Whatman no 1 (125mm) filter paper. The filtrates were concentrated using rotary evaporator and the extract stored in the refrigerator at 4°C (Patrick and Chidiebere, 2016).

Chemicals

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

Phytochemical analysis

Phytochemical tests were carried out on the aqueous, ethanol, methanol and n-hexane extracts using standard phytochemical methods as described by Harbone (1973), Sofowara (1993), Treases and Evans (1989). The phytochemicals that were assayed include alkaloids, cardiac glycosides, cyanogenic glycosides, flavonoids, saponin, Tannins and Phenols.

Experimental animals

Male wistar albino rats (10-12 weeks) weighing between 120 and 150g obtained from the animal house of Chris Research farm, Awka were used for the study. The animals were housed in the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The animals were allowed free access to rat feed and water during the experiment. They were acclimatized for two weeks before the commencement of the experiment. The animals were monitored daily and the cages cleaned at three (3) days interval.

Acute toxicity (LD_{50}) evaluation

The median lethal dose (LD_{50}) for each of the extracts were determined using Lorke's method (1983). Twelve male rats were used for the determination of the median lethal dose for each extract. The twelve (12) rats were randomized into six groups; three rats each for 10, 100 and 1000mg/kg bw and one rats each for 1600, 2900 and 5000mg/kg bw. The animals were monitored for changes in behaviour and mortality within 2 hrs, 24 hrs and 14 days after a single administration of the extracts.

Determination of the Impact of the extracts on Haematological and Biochemical Parameters

A total of twenty-five (25) male wistar albino rats were randomized into five (5) groups of five rats each and used for the study. Group A was the experimental control, Group B, C, D and E were treated with 200mg/kg bw of aqueous, ethanol, methanol and n-hexane extract respectively for a period of twenty-eight (28) days. At the end of the 28 days treatment the rats were anesthesized and blood was collected by cardiac puncture and put in an EDTA bottle and plain tubes for haematological and biochemical analysis respectively.

Haematological analysis

Haematological parameters were determined using automated auto haematology analyzer (Mindray-BC-28000). The haematological parameters that were analyzed include packed cell volume (PCV), haemoglobin (HGB), white blood cell (WBC), Red blood cell (RBC) and Platelets.

Biochemical analysis

Liver function test

Serum biochemical indices routinely estimated for liver functions including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Bilirubin were determined using Randox diagnostic kits (Jendrassik and Grof, 1938; Reitman and Frankel, 1957). The procedure

used was according to the manufacturer's instruction.

Kidney function test

Kidney function parameters Na^+, Cl^-, K^+ and HCO_3 were measured by autoanalyser, Selectra Junior manufactured by Vital Scientific B. V. Netherlands. Urea and creatinine were analysed using Randox diagnostic kits (Chaney and Marbach, 1962; Bartels and Bohmer, 1972). The procedure is according to the manufacturer's instruction.

Statistical analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All data were expressed as mean ± standard deviation.

Analysis of significant differences were done by one-way analysis of variance (ANOVA) to compare the control and the treatment groups followed by Duncan Multiple Method. Differences at $p< 0.05$ were considered as significant.

Results

The qualitative phytochemical screening of the aqueous, ethanol, methanol and n-hexane extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves revealed the presence of important phytochemicals (Table 1).

Table 1. Phytochemical analysis of a combination of leaf extracts of *Telfairiaoccidentalis* and *Mucunapruriens* (1:1).

Phytochemicals	Aqueous Extract	Ethanol Extract	Methanol Extract	n-Hexane Extract
Alkaloids	+	+	+	++
Cardiac glycosides	+	+	+	-
Cyanogenic glycosides	-	-	-	-
Flavonoids	++	+	++	+
Saponins	+++	+++	++	+
Tannins	++	+	+	+
Phenols	+	+	+	-

+ Present ++ moderately present +++ Present in sufficient Amount - Not present.

The aqueous leaf extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* revealed the presence of saponins in substantial amount. Flavonoids and tannins were moderately present while alkaloids, cardiac glycosides and phenols were detected in very minute amount.

The ethanol leaf extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* revealed the presence of saponins in substantial amount.

Alkaloids, cardiac glycoside, flavonoids, tannins and phenols were present in minute quantities.

Table 2. Result of the median lethal dose (LD_{50}) of a combination of leaf extracts of *Telfairiaoccidentalis* and *Mucunapruriens* (1:1).

Combination of Leaf extract of *Telfairiaoccidentalis* and *Mucunapruriens* (1:1)	LD_{50} (mg/kg)
Aqueous extract	3808
Ethanol extract	3808
Methanol extract	3808
n-Hexane extract	3808

The methanol leaf extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* reveal the presence of flavonoids and saponins in moderate amount while alkaloids, cardiac glycosides, tannin and phenols were detected in minute quantities. The n-hexane leaf extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* reveal the presence of alkaloids in moderate amount while

flavonoids, saponins, tannins and phenols were detected in minute quantities. Cyanogenic glycosides were not detected in all the extracts analysed. The result of the fourteen-days median lethal dose (LD_{50}) of the aqueous, ethanol, methanol and n-hexane extracts of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves revealed that the LD_{50} of the extracts was 3808mg/kg bw (Table 2).

The effect of continuous administration of the aqueous, ethanol, methanol and n-hexane extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves (50:50) on haematological parameters of male rats was represented in Table 3.There was a significant ($p<0.05$) increase in the packed cell volume, haemoglobin, red blood cells and platelet count of the rats treated with 200mg/kg bw of aqueous, ethanol and methanol extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves compared with the experimental control. The rats treated with the n-hexane extract did not show any significant ($p<0.05$) difference in their packed cell volume, haemoglobin, red blood cells and platelet count compared to the experimental control. The rats that were treated with 200mg/kg bw of aqueous, ethanol and methanol extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves did not show any significant ($p<0.05$) difference in their white blood cell counts compared to the experimental control but the white blood cell count of the rats treated with n-hexane extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves significantly ($p<0.05$) increased compared with the experimental control.

Table 3. Effect of treatment with the extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves (1:1) on haematological parameters of male rats.

Treatment groups	PCV (%)	HGB (g/dl)	WBC (x 10^9/L	RBC (x10^{12}/L)	Platelets (x10^9/L)
Control	33.12±2.90	10.80±0.20	15.75±0.10	5.143±0.03	326.5±4.08
Aqueous extract (200mg/kg)	39.39±0.43*	12.62±0.32*	16.09±0.10	6.023±0.05*	328.7±3.21*
Ethanol extract (200mg/kg)	40.57±3.04*	13.03±0.15*	15.98±0.40	7.532±0.03*	370.1±2.65*
Methanol extract (200mg/kg)	39.53±1.71*	13.02±0.31*	15.09±1.23	7.428±0.04*	335.4±2.73*
n-hexane extract (200mg/kg)	32.75±2.38	10.67±0.22	20.23±0.30*	4.638±0.02	286.5±3.50#

* Significant increase at $p<0.05$ treatment compared with the experimental control # Significant decrease at $p<0.05$ treatment compared with the experimental control.

The effect of treatment with the aqueous, ethanol and methanol extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves did not show any significant ($p<0.05$) difference in their aspartate aminotransferase enzyme, alanine aminotransferase enzyme, alkaline phosphatase enzyme, total bilirubin and direct bilirubin level compared to the experimental control (Table 4). There was a significant ($p<0.05$) increase in the aspartate aminotransferase, total bilirubin and direct bilirubin level of the rats treated with the n-hexane extract compared with the experimental control. However, there was no significant ($p<0.05$) difference in the alanine aminotransferase and alkaline phosphatase of the rats treated with n-hexane extract compared with the experimental control. Treatment with the aqueous, ethanol and methanol extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves did not show any significant ($p<0.05$) difference in their Na^+, Cl^-, K^+, urea and creatinine levels compared to the experimental control (Table 5).

There was a significant ($p<0.05$) increase in the bicarbonate, urea and creatinine level of the rats treated with the n-hexane extract compared with the experimental control. However, there was no significant ($p<0.05$) difference in the potassium ion, chloride ion and sodium ion levels of the rats treated with n-hexane extract compared with the experimental control.

Discussion

Important phytochemicals were detected in the leaves of *Telfairia occidentalis* and *Mucuna pruriens* which

may be responsible for its medicinal and pharmacological activities. Plants use Phytochemicals as a defence against infections and diseases. Secondary metabolites are synthesized by the plants as part of the defense system of the plant (Phan *et al.*, 2001). Flavonoids play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules (Atmani *et al.*, 2009). In medicine, tannins are used as antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity and anticancer actions (Wink *et al.*, 1998). Saponins have been observed to be antioxidants and equally act as antifungal and antiviral (Morrissey and Osbourn, 1999; Traore *et al.*, 2000). Acute toxicity causes severe disruption of body function that can lead to death within 14 days. The LD_{50} value of the extracts suggest that the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves were not toxic. According to Lorke (1983) method any death recorded as a result of administration of 5000mg/kg bw suggest that the plant extract is not toxic.

Table 4. Effect of treatment with extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves (1:1) on liver function parameters of male rats.

Treatment groups	AST (U/L)	ALT (U/L)	ALP (IU/L)	T. Bilirubin (mg/dl)	D. Bilirubin (mg/dl)
Control	28.26±0.81	24.76±0.92	45.34±1.23	1.062±0.02	0.531±0.01
Aqueous extract (200mg/kg)	27.54±1.30	23.93±0.54	43.85±1.05	1.434±0.03	0.418±0.01
Ethanol extract (200mg/kg)	28.50±0.74	23.54±0.53	44.26±1.95	1.731±0.02	0.527±0.02
Methanol extract (200mg/kg)	28.39±2.84	24.05±1.43	44.60±1.43	1.084±0.25	0.513±0.03
n-hexane extract (200mg/kg)	29.65±1.35*	25.23±0.87	46.17±1.74	2.023±0.02*	0.634±0.01*

* Significant increase at $p<0.05$ treatment compared with the experimental control # Significant decrease at $p<0.05$ treatment compared with the experimental control.

Results of haematological studies are useful in the diagnosis and management of some diseases as well as investigation of the extent of damage to blood (Onyeyili *et al.,* 1992; Togun *et al.,* 2007). The results of the haematological analysis carried out on the rats fed with aqueous extracts of *Mucuna pruriens* leaves revealed that the extracts boosted blood production (Obioma *et al.*, 2014). However, although there is a report on the effect of aqueous extract of M. pruriens on the haematological parameters of rats, no research has been carried out on the synergistic effect of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leave extracts on haematological and biochemical parameters of wistar albino rats.

Red blood cells serve as a carrier of haemoglobin and this haemoglobin reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris, 1996; Chineke *et al.*, 2006). According to Isaac *et al.* (2013) Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume shows a better transportation of oxygen. Packed Cell Volume and haemoglobin are major indices for evaluating circulatory red blood cells, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells (Awodi *et al.*, 2006; Chineke *et al.,* 2006).

The aqueous, ethanol and methanol extract did not cause any disruptive action on the liver as seen from the levels of the alanine transaminase, aspartate transaminase and alkaline phosphatase of the rats treated with these extracts for a period of twenty-eight days. These results suggest that the combination of leaf extract of *Telfairia occidentalis* and *Mucuna pruriens* modulates the liver function favourably without causing any known damage to the liver. High levels of AST and bilirubin as seen from the rats administered n-hexane extract is an indication of hepatic-toxicity. This is in line with the report in another study by Anofi *et al.*, (2012).

Treatment with the aqueous, ethanol and methanol

extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves did not show any significant ($p<0.05$) difference in their Na^+, Cl^-, K^+, urea and creatinine levels compared to the experimental control.

There was a significant ($p<0.05$) increase in the bicarbonate, urea and creatinine level of the rats treated with the n-hexane extract compared with the experimental control. Increased levels of bicarbonate, urea and creatinine can be an indication of toxicity to the kidney. Creatinine is a metabolic waste released into the blood from the breakdown of a high energy molecule, phosphor creatinine. High levels of creatinine can mean spontaneous metabolism of phosphocreatine which is an indication of malfunction of the kidney (Adepoju and Odubena, 2009).

Table 5. Effect of treatment with extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves (1:1) on kidney function parameters of male rats.

Treatment groups	K^+ (mmol/L)	Cl^- (mmol/L)	Na^+ (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Control	7.821±0.13	101.2±3.76	143.5±5.98	26.72±0.53	10.32±0.02	34.87±1.09
Aqueous extract (200mg/kg)	7.870±0.43	101.5±1.45	142.3±3.86	26.04±0.87	9.607±0.06	35.27±1.43
Ethanol extract (200mg/kg)	7.769±0.54	100.6±1.90	143.1±3.84	27.26±1.54	10.40±0.75	34.53±1.61
Methanol extract (200mg/kg)	7.787±0.82	100.9±2.89	142.6±4.73	25.83±1.05	8.93±0.54#	34.08±0.98
n-hexane extract (200mg/kg)	7.341±0.53	101.3±1.62	142.7±4.31	28.95±0.76*	12.71±0.05*	36.39±2.36*

* Significant increase at $p<0.05$ treatment compared with the experimental control # Significant decrease at $p<0.05$ treatment compared with the experimental control.

The study reveals that the aqueous, ethanol and methanol extracts of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves increased the packed cell volume, red blood cells, haemoglobin and platelet count without altering the essential biochemical parameters. The n-hexane extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves significantly altered the haematological and essential biochemical parameters. It is of interest to note from the results that n-hexane extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves cannot be used favorably as a blood booster or for replenishing blood in anaemic patients as it can lead to toxicity as revealed from the result of the biochemical analysis.

Further study is needed to determine the effect of these extracts of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves on male wistar albino rats after a prolonged administration for a period of about three months. Also, it will be necessary to further determine the effect of the administration of the aqueous, ethanol and methanol extract of the combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves on the histology of the liver, kidney and lungs to know whether it can cause organ damage or toxicity.

Conclusion

The results obtained from the haematological analysis led to the conclusion that the aqueous extract, ethanol extract and methanol extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* can significantly boost blood production without altering the essential biochemical parameters.

This suggest that the extracts can be used to treat anaemia due to its haemoglobin boosting properties. However, the n-hexane extract of a combination of *Telfairia occidentalis* and *Mucuna pruriens* leaves caused a significant increase in the wbc of the animals suggesting that it is not a good substitute to be used as blood booster. The n-hexane extract also increased the essential biochemical parameters studied suggesting that it may be toxic.

Conflict of interest

Authors hereby declare no conflict of interest.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria in accordance with the Institutional Animal Care and Use Policy in Research, Testing and Education.

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