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Comparative effects of ethanolic extracts of *Ficus carica* and *Mucuna pruriens* leaves on haematological parameters in albino rats

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ABSTRACT: The comparative effects of the ethanolic extracts of *Ficus carica* and *Mucuna pruriens* on haematological parameters were investigated in albino rats. The animals were divided into three main groups: group 1 which served as the control, received 5.0ml/kg body weight of normal saline, while groups 2 and 3 received a daily administration (per os) of 200mg/kg body weight of extracts of *M. pruriens* and *F. carica* respectively for 14 days. Results showed that the extracts significantly increased the haemoglobin concentration, PCV and red blood cell count by the 14th day when compared with the control ($p < 0.05$). *F. carica* was found to be more effective than *M. pruriens* in elevating the red blood cell count, especially by the 14th day. The two extracts, however, significantly decreased the total white blood cell count, as well as the percentage neutrophils, when compared with the control group ($p < 0.05$), but not significant between test groups, even by the 14th day. Phytochemical analyses showed the presence of alkaloids, flavonoids, saponins, cardiac glycosides and carbohydrates in both plants. Tannins were present in *F. carica* but not in *M. pruriens*. These results thus justify the ethnobotanical use of these plants as blood building herbs.

Key words: *Mucuna pruriens*, *Ficus carica*, haematological parameters

Introduction

The importance of blood in maintaining good health cannot be overstated. The Chinese describe blood as the 'mother of energy' in the sense that it provides the basic building materials and fluid substances that are required to nourish the life essence of our being; thus blood is represented as a receptacle for sustaining our life energy (Sheng, 2003). The functions of blood are many and varied. Besides providing material nourishments, the blood also provides the necessary moisture needed by the internal organs to function properly. Insufficient blood or blood deficiencies can cause many problems ranging from weakness, lethargy, inability to concentrate, hot flushes, increased susceptibility to infection, shortness of breath, fatigue, dizziness, palpitation, anxiety, depression, insomnia, nervousness, headache and diminished sex drive. Women in particular, are especially susceptible to blood deficiencies due to their monthly menstrual cycle. In addition, because the life span of the red blood cells is relatively short, the blood needs to be constantly replenished (Sheng, 2003).

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In Nigeria, the local people are known for using natural herbs and herbal formulae for addressing various kinds of blood deficiencies. In south-eastern Nigeria, the leaves of *Mucuna pruriens* and *Ficus carica*, among others, are considered excellent natural herbal blood boosters, used especially for debilitating conditions, acute blood loss and blood deficiency diseases (Obadoni and Ochuko, 2001).

Mucuna pruriens (L.) is an annual climbing legume that grows 3-18 m in height, indigenous to tropical regions, especially Africa, India, and the West Indies. It is wide spread over most of the subcontinent and is found in bushes, hedges, and dry-deciduous, low forests throughout the plains of India (Sastry and Methrotas, 1991).

Mucuna pruriens belongs to the family Fabaceae. Some other common names include: Common Cow-itch, Konch, mucuna, nescafé, pó de mico, fava- coceira, cabeça-de-frade, cowage, cowhage, Bengal bean, Mauritius bean, itchy bean, krame, picapica, chiporro, and buffalo bean. The roots are bitter, sweet, thermogenic, emollient, stimulant, purgative, aphrodisiac and diuretic. The leaves are also aphrodisiac. The seeds are astringent, laxative, anthelmenthic, alexipharmic and tonic (Taylor, 2005). A clinical study confirmed the efficacy of the seeds in the management of Parkinson's disease by virtue of their L-DOPA content (Manyam et al., 2004; Bell et al., 1971).

M. pruriens has been shown to increase testosterone levels (Amin et al., 1996), leading to deposition of protein in the muscles and increased muscle mass and strength (Bhasin et al., 1996). The itch-producing property and resulting blisters and dermatitis of *M. pruriens* is attributed to the trichomes (hair) present on the pods. It has been established that this unique property is accounted by the presence of 5-hydroxy tryptamine (5-HT) and mucunian in the hair (Armstrong et al., 1953, Saltry, 1990). Some reports show that anti-histaminics afford protection against the itch (Broadbent, 1953). The antimicrobial activity (against gram positive, gram negative and spore forming bacteria and also fungi) of the methanol extract of the leaf of *M. pruriens* has been reported. Some of the medicinal properties attributed to the plant include that the roots are thermogenic, antihelminthic, and also used to relieve constipation, neuropathy and ulcers (Warrier et al, 1996).

The leaves of *M. pruriens* are also used in the management of ulcers, cephalgia and general debility. The seeds have been known to contain large amounts of proteins and minerals and with high calorific value, but also with high levels of antinutritive properties such as phenolics, tannins, L-dopa, trypsin inhibitors, and phyto haemagglutinins (Vadivel and Janardhanan, 2000). The seed also contains glutathione, lecithin, gallic acid, nicotine, prurenidine (Rastogi and Kavathekar, 1991), and 4-tetra isoquinoline alkaloids (Misra and Wagner, 2004), and as such are used as astringent, laxative, antihelminthic, aphrodisiac and tonic. L- dopa, found in the cotyledon of the seed, was found to increase the brain mitochondria complex – 1 activity and thus has been attributed to the neurorestorative effects; as such it has been useful in the management of Parkinson's disease (Manyam et al, 2004; Barclay, 2004). The seed powder also has hypoglycaemic (Agharkar, 1991; Akhtar et al, 1990), anti snake venom activity by activation of prothrombin (Guerranti et al, 2002) and anti oxidant properties (Tripathi and Upadhyay, 2002). Much work has not been done on the haematological effects of the leaf, hence one of the main reason of this investigation.

Ficus carica (or fig, family-Moraceae) is a tree of small dimension that produces copious milky latex and 'fruits', which are actually a synconium, i.e. a fleshy hollow receptacle with a small opening at the apex partly closed by small scales. The latex of the unripe fruit and other parts of the tree cause severe irritation to the skin, if not removed promptly. The photosensitizing properties are related to forocoumarins as the active principle. The latex is widely applied on warts, skin ulcers, sores and abnormal growth (Bohlooli et al, 2007). It is also taken as purgative, vermifuge and antihelminthic, but with considerable risk (De-Amorin et al, 1999). The leaf decoction has been used as a remedy for diabetes and calcification of the liver and kidneys. It also lowers the levels of total cholesterol, triglycerides and total cholesterol/HDL-cholesterol ratio (Perez et al, 1999a, b; Canal et al, 2002; Asadi et al, 2006; Fatemi et al, 2007). In addition, *F. carica* has been used to treat many other medical conditions such as cough, flu, asthma, cancer, abscesses, constipation, diabetes and gingivitis (Serraclará et al, 1998; Rubnov et al, 2000; Gilani et al, 2008). The plant is used locally in South-East Nigeria for blood building purposes.

The aim of this present study was therefore to compare the effects of ethanolic extracts of the leaves of these two plants on haematological parameters. The extracts were also subjected to phytochemical analyses.

Materials and Methods

Plant Materials:

Fresh leaves of *M. pruriens* and *F. carica* were collected from the Science Village of the Nnamdi Azikiwe University, Awka, Anambra State and authenticated by the third author, of the Department of Botany, Faculty of Natural Sciences of the University. Voucher specimen were deposited at the herbarium of the University.

Preparation of plant extract:

The leaves were washed, air – dried and extracted with 70% ethanol (v/v) by cold extraction for 48 hours. The extracts were evaporated to dryness at 40°C in a water bath.

Animals:

Healthy albino rats of both sexes (average weight-120g), obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were divided into three main groups: group 1 served as the control, which received 5.0ml/kg body weight of normal saline, while groups 2 and 3 received a daily administration (per os) of 200mg/kg body weight of extracts of *M. pruriens* and *F. carica* respectively for 14days. The animals were also allowed rat chow and water *ad libitum*.

Collection of blood samples

Blood samples were collected by cardiac puncture after days 4, 8 and 14 days of receiving the extracts and stored in anticoagulant bottles containing EDTA. The blood samples were then used subsequently for haematological analyses.

Haematological analyses:

Determination of Red blood cell count (with improved Neubauer chamber):

0.02ml of the anticoagulated blood was diluted to 4.0ml (i.e. 1:200 dilution). The counting chamber was charged to obtain Newton's ring that gives a rainbow effect on both sides. The Neubauer chamber is then loaded with samples and allowed to stay for 20minutes. The cells were counted using the microscope on the four square corners and the number multiplied by 1000 to obtain the actual values.

Estimation of haemoglobin concentration:

0.02ml of the blood was introduced into 5.0ml of Drabskin's solution (1: 2500 in a test tube and allowed to stay for 5 minutes for full colour development. The optical density (OD) was then read using a colorimeter with green filter, with Drabskin solution as blank. A standard solution of haemoglobin was prepared and the OD of standard was used to calculate the concentration of the test haemoglobin as follows:

$$\frac{\text{OD of test}}{\text{OD of Standard}} \times \text{Concentration of test haemoglobin solution}$$

The packed cell volume (PCV) or the haematocrit value was obtained by multiplying the percentage haemoglobin by a factor of 3, i.e.

$$\text{PCV (\%)} = \text{Hb (\%)} \times \text{factor 3}$$

Determination of total white blood cell (WBC) count with improved Neuber chamber

0.02ml of the blood sample was added to 0.38ml of Turk's solution (1:20 dilution) in a test tube. The chamber was loaded with the diluted blood and allowed to stay for 5 minutes. The WBC were counted and multiplied by 50 to give the total count.

Determination of differential white blood cell count

A thin film of the blood was made on a slide and allowed to air dry. The Leishmann stain was added and allowed to stay for 2 minutes, then diluted with a buffer and allowed to stay for 8 minutes. The slides were washed with the same buffer and allowed to air dry. A drop of immersion oil was added and the slides were viewed under the microscope (x 100 magnification). A total of 100 white blood cells were counted and differentiated into types.

Phytochemical analyses

The extracts were subjected to phytochemical analyses according to the methods of Harbone (1973).

Results and Discussion

Phytochemical screening:

The results of the phytochemical screening of the leaf extract of the two plants are summarized in table 1. Alkaloids, flavonoids, saponins, cardiac glycosides and carbohydrates were present in both plants. Tannins were present in *F. carica* but not in *M. pruriens*, although the presence of tannins was reported in the seeds by Vadivel and Janardhanan, (2000). Some of the biological functions of flavonoids include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors (Okwu, 2004). Saponins, on the other hand, have the properties of precipitation of proteins, cholesterol-binding and haemolysis. Other phyto components such as alkaloids and glycosides found in these plants also do not have properties relating to increased haematopoiesis.

Haematological analyses

Figs 1a-e show the haematological changes in the animals fed with the extracts for 14 days. Time-dependent increases in haemoglobin, red blood cells and PCV implies that the extracts may enhance the populations of red blood cells produced from the bone marrow, as well as increased the oxygen-carrying capacity of the whole blood because of the increased number of red blood cells in the blood. These increases were not significantly different between the two extracts and with the control at $p < 0.05$, except by the 14th day. *Ficus carica* appeared to be more effective than *M. pruriens* for the red blood cell level as observed by the 14th day. It is noteworthy that there is absence of tannins in the extracts of *M. pruriens*, which may be a contributing factor. *F. carica* on the other hand has been reported to have huge amounts of flavonoids (Fatemi et al, 2007).

Statistically significant increases in platelet count and PCV were reported with the seed extract of *M. pruriens* (Adepoju and Odubena, 2009). Both extracts also reduced total WBC as well as the neutrophil count in a significant manner when compared with control, but not significantly different between test groups ($p < 0.05$). It is probable that the extract may have caused destruction or impaired production of white blood cells, probably by affecting the production of regulatory factors involved in haematopoiesis. However, *M. pruriens* has been shown to have anti oxidant properties which could equally suppress production of white blood cells (Okwu, 2004). It has been reported that the granulocyte/macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells (Golde and Fumimaro, 1975; Metcalfe, 1985); thus chronic administration of these extracts for prolonged periods may in fact suppress proliferation, thereby predisposing the animals to infection.

Conclusively, in spite of the popularity of these plants as herbal blood boosters, in that they enhance the haemopoietic system; the impaired white blood cell production will definitely limit their use. Further research on their phytochemical constituents and the identification of the component responsible for the observed leucopenia is worth investigating.

Table 1: Phytochemical Analyses

Phytoconstituent	<i>M. pruriens</i>	<i>F. carica</i>
Alkaloids	++	+
Saponins	++	++
Flavonoids	++	++
Glycosides	++	++
Carbohydrates	+	+
Tannins	-	++

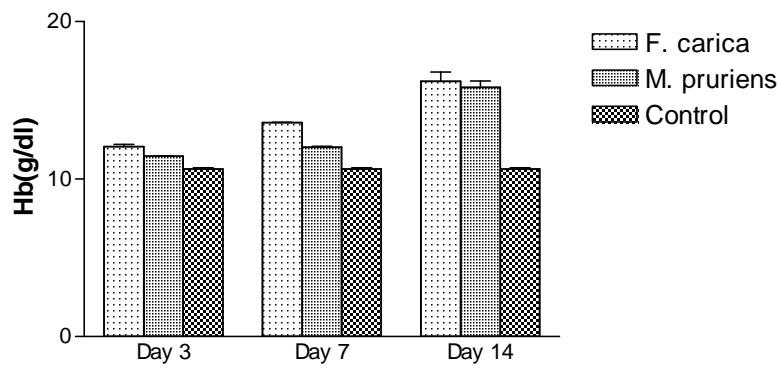


Fig. 1a: Effect of extracts of *F. carica* and *M. pruriens* on haemoglobin level.

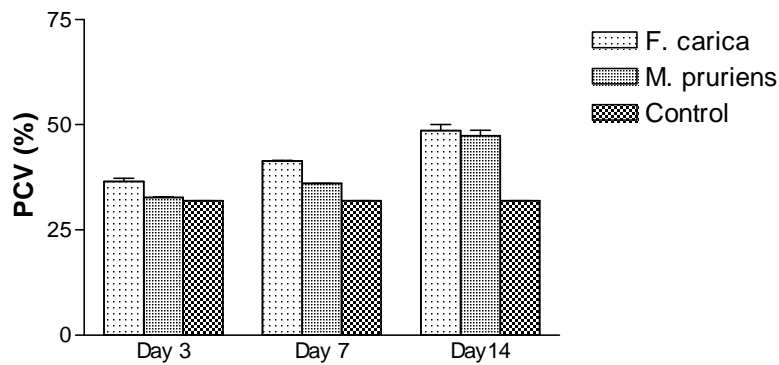


Fig. 1b: Effect of extracts of *F. carica* and *M. pruriens* on packed cell volume.

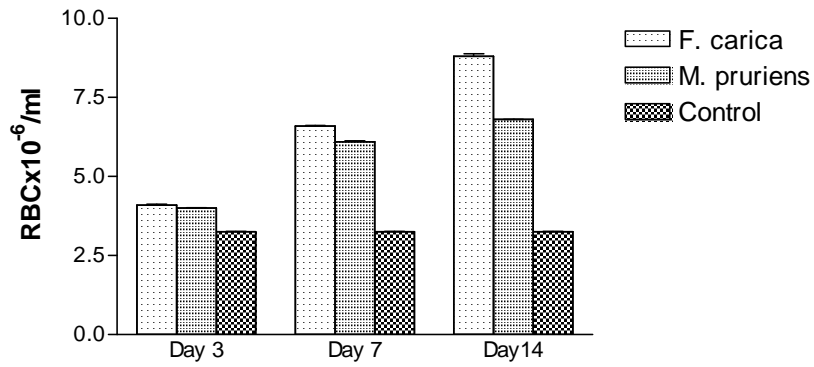


Fig. 1c: Effect of extracts of *F. carica* and *M. pruriens* on red blood cells.

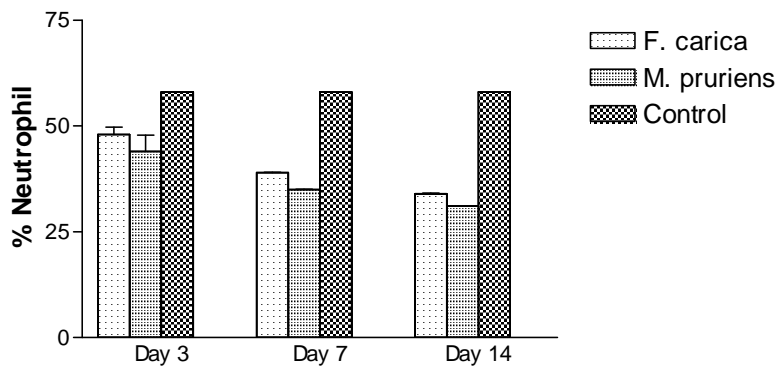


Fig. 1d: Effect of extracts of *F. carica* and *M. pruriens* on percentage of neutrophils.

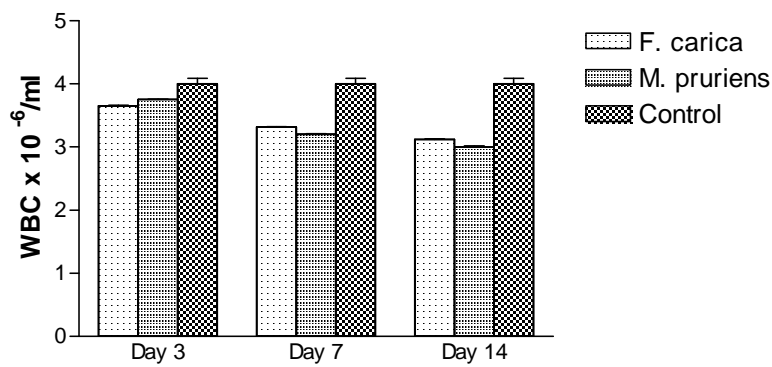


Fig. 1e: Effect of extracts of *F. carica* and *M. pruriens* on total white blood cell count.

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