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Original Research

Physicochemical properties of Mucuna pruriens seed oil (MPSO), and the toxicological effects of a MPSO-based diet

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ABSTRACT: The toxicological effects of *Mucuna pruriens* oil based diet were evaluated and compared with that of palm oil diet for 28 days. The physico-chemical analysis of the *Mucuna pruriens* oil showed that it has a moisture content of 7.85%, oil yield of 6.00%, pH of 5.65, density of 0.39, iodine value of 24.40/100g fat, acid value of 51.40mg NaOH/g, peroxide value of 0.10mEq/Kg, saponification value of 86.05mg/KOH/g, free fatty acid value of 0.40mg/dl, viscosity of 37.54 and unsaponifiable matters of 46.10. The aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities were significantly ($p<0.05$) increased in rats fed *Mucuna pruriens* oil meal compared to the palm oil group. The serum total and conjugated bilirubin, total proteins, albumin, creatinine and urea concentrations were also significantly ($p<0.05$) increased in the test group. Histological examination of the rat organs revealed the presence of lesions, tubular atrophy and mild oedema on organs from the test group. This is an indication that *Mucuna pruriens* oil is not completely safe for consumption.

KEYWORDS: *Mucuna pruriens*, toxicological effect, histology, phytochemical.

INTRODUCTION

Oil crops and their products have become very popular in the world today. *Mucuna pruriens* which belongs to the family of *Fabaceae* is an oil crop which has not been sufficiently put into use (Rajeshwar *et al*., 2005). It is commonly known as cowhage, velvet bean and fogarate. In south eastern Nigeria, it is known as 'Agbala' while in Yoruba, it is known as "Yerepe". A major characteristic of this plant is its ability to cause extreme itchiness when touched. (Rajeshwar *et al*., 2005). The pods contain seeds that are black or white. *Mucuna pruriens* is traditionally used as food, feeds and in pharmaceuticals (Sridhar and Bhat, 2007). The pods and leaves are also used as vegetables in some ethnic groups in Nigeria (Adebowale and Lawal, 2003). Some rural communities in Enugu state consume the seeds during famine or scarcity of food (Onweluzo and Eilitta, 2003). The seeds *of M. pruriens* are also used as soup thickeners (Ukachukwu *et al*., 2002). Thomas (2006) reported the anti-inflammatory, diuretic, antihelmintic, antihypertensive and cough suppressant properties of *M. pruriens*. Siddhuraju *et al.,* (1996) reported that velvet bean contains toxic substances. Despite the varied uses of *M. pruriens* seeds, there is dearth of information on the possible toxicological implications of consumption of *mucuna pruriens* oil and this has necessitated this work.

MATERIALS AND METHODS

Preparation of Sample

The dry *Mucuna pruriens* seeds were oven dried at 40^{o}C for 60 minutes, after which the seeds were dehulled to separate the seed coat from the cotyledon. The seed coat was discarded and the cotyledon ground into powder with the aid of a hand mill.

Oil Extraction

N-hexane was used for the extraction of oil from the ground seeds. Five hundred grammes of dried *M. pruriens* seeds were ground, soaked in n-hexane and allowed to stand for 24 hours at room temperature. After 24 hours, the samples were thoroughly shaken and filtered using whatman No.1 filter paper. The filtrate was transferred into a round bottomed flask, thereafter, soxhlet apparatus was affixed to the round bottomed flask and refluxing was carried out for 4hrs. The solvent was then recovered after heating while the oil remained in the round bottom flask. The round bottomed flask with the content was brought out and dried to a constant weight in an oven. The oil was cooled in a dessicator, turned into a laboratory sample bottle and labelled (Laximinatrain and Hildebert, 2007).

Determination of Fatty Acid Composition

Potassium hydroxide prepared in 2 N of methanol was added to one gramme of the oil sample to saponify the oil. The oil was emulsified by addition of prepared Conc. HCl with methanol (1.4) in a soap solution. This was followed by addition of n- heptane to select the oil. Normal saline was added to salt out the outer composition and 0.5 μl syringe was used to measure out and transfer to a Gas-Liquid chromatography equipment. After 10 minutes, the fatty acids were identified in a graph, having peaks, with their area and carbon number.

Composition of *Mucuna pruriens* Oil Based Treatment Diet

The treatment diet was composed of 1 g of vitamin, 10 g of *M. pruriens* oil, 26 g of casein as protein source, 5 g of corn starch as carbohydrate source and 4 g of salt mixture. The control diet had a similar composition but had 10 g of palm oil instead of *M. pruriens* oil (Table 3).

Animals

Adult male wistar albino rats (*Rattus norvegicus*) used in the study were 8-10 weeks old and weighed 150±10 g. They were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in well ventilated stainless steel cages and acclimatised for one week.

Determination of Physico-chemical parameters

The physico-chemical parameters were determined by the method of (AOAC, 1997).

Sub-acute toxicity test

Male rats were completely randomised into two groups of five rats each. Group 1, (test) received diet with 10% *Mucuna pruriens* oil as a source of fat. Group 2, (control), received casein based diet with 10% palm oil as the source of fat. They were allowed free access to the feed and water daily.

The rats were sacrificed by cervical dislocation after 28days daily feed and water administration. The blood was collected into sterile sample bottles. It was allowed to clot, centrifuged at 10,000 rpm and the serum collected for the analyses. Portions of the liver and kidney were collected into plastic containers containing formal saline.

Biochemical assay

The concentration of serum total protein was evaluated photometrically using QCA Test kit, by the method of Wiechselbum (1946). Albumin concentration was determined using the randox bioassay test kit by the method of Grant (1987). Alkaline phosphatase (ALP) activity was assayed using the method described by King and King (1954). Alanine aminotransferase (ALT) and aspartate aminotranferase (AST) activities were assayed using the method of Reitman and Frankel (1957). Total and conjugated bilirubin concentrations were determined by the method described by Jendrassik and Grof (1938). The concentration of urea was dertermined by the method of Chaney and Marbach (1962), and creatinine concentration was determined using the method of Taussky (1961).

Histopathological Studies

Histopathological studies were determined by the method described by Bancroft and Stevens (1999). Sections of the different organs from each group were collected in a sterile universal container containing 10 % neutral buffered formalin for 24 hours. The tissues were dehydrated through graded series of ethanol (50%, 70%, 90% and 2 times of 100% ethanol) for complete dehydration, cleared in xylene to render the tissue transparent by removing ethanol from dehydrated sections and embedded in paraffin wax to provide a hard support for sectioning. The blocks were sectioned in the transverse plane at 7 µm using LEICA microtome. Every third section was mounted on the glass slide and stained with haematoxylin and eosin. A drop of distrene tricresyl phosphate xylene mountant devoid of air bubbles was place on the slide and cover slips were carefully placed over the slide. Photomicrographs of selected sections were captured using Motic 2001 camera (Motican, UK) attached to a microscope at ×400.

Statistical analysis

The results obtained from the laboratory experiment were analysed using the Statistical Package for Social Sciences (SPSS) version 18.0. Analysis of variance (ANOVA) and student's t-test were used to compare means, and values were considered significant at $P \leq 0.05$. Post hoc multiple comparisons for differences between groups were performed by least significance difference (LSD). All the data are expressed as mean ± standard deviation (SD).

RESULTS

The physico-chemical analyses of *M. pruriens* oil were shown in Table 1. The physico-chemical analysis of the *M. pruriens* oil showed a moisture content of 7.85±0.10%, oil yield of 6.00±0.01%, pH of 5.65±0.10, density of 0.39±0.02, iodine value of 24.40±/1.00/100 g fat, acid value of 51.40 ±0.05 mg NaOH/g, peroxide value of 0.10±0.23 mEq/Kg, saponification value of 86.05 ±0.10mg/KOH/g, free fatty acid value of 0.40±0.60 mg/dl, viscosity of 37.54±0.67 and unsaponifiable matters of 46.10±0.41.

Table 1: The physico-chemical properties of *Mucuna pruriens* seed oil

Colour	Ember yellow
Moisture	7.85±0.10%
Melting temperature	24.40 °C
Oil yield	6.00±0.01%
pH	5.65±0.10
Density	0.385±0.02
Iodine value	24.40±1.00 per 100g fat
Acid value	51.40±0.05 mg NaoH/g
Peroxide value	0.10±0.23 mEq/Kg
Saponification Value	86.05±0.10 mg/KOH/g
FFA value	0.40±0.60 mg/dl
Viscocity	37.54±0.67
Unsaponifiable matters	46.10±0.41

The fatty acid profile of *M. pruriens* oil is shown in Table 2. The data showed that the oil contains both saturated (myristic 7.18%, palmitic 9.87%, stearic 13.59%) and unsaturated (myristoleic 8.53%, oleic 14.62%, linoleic 16.82%) fatty acids.

The activities of ALP, AST and ALT were significantly ($p<0.05$) increased throughout the experiment (Figure 1). There were significant ($p<0.05$) increases in the serum total protein, albumin, total and conjugated bilirubin contents of the animals (Figures 2 and 3). Serum urea and creatinine contents of the animals were significantly ($p<0.05$) increased (Figure 4).

Table 2: Fatty acid profile of *Mucuna pruriens* Oil Based

Fatty acid	Percentage(%)
Myristic acid	7.18
Palmitic acid	9.87
Stearic acid	13.59
Myristoleic acid	8.53
Oleic	14.62
Linoleic	16.82

Table 3: Composition of *Mucuna pruriens* Oil Based Treatment Diet

g/100g diet	Components
1	Vitamin mixture
10	Mucuna oil/palm oil
26	Casein (protein)
59	Corn starch (carbohydrate)
4	Salt mixture

DISCUSSION

The nutritional composition and acclaimed medicinal values of a number of oils has attracted their use domestically and industrially. This has lead to exploration of new and unexploited crops that are capable of producing fats and oils. These oils may contain bioactive principles with potentials to cause adverse effects.

The physico-chemical analyses of *M. pruriens* oil revealed that the oil is amber yellow in colour, which suggests that it may contain the antioxidant β-carotene. The pH value indicates that the oil is acidic, inferring a good nutritional quality. Iodine value is an index of the degree of unsaturation and susceptibility of oil to oxidation (Bello *et al.*, 2011). The iodine value of *M. pruriens* oil obtained in this study showed that it is a non-drying oil and could be useful in the manufacture of soap (Kochhar, 1998).

The percentage moisture content (7.85%) of *M. pruriens* oil in this study is in the same range with the report of Thomas (2006) who reported 10%. The percentage oil yield of 6.0% corresponds with the values (5.7%, 6.3%, and 7.6 %) reported in earlier studies by (Njoku, 1997; Thomas 2006; Sridhar and Bhat, 2007). This may be due to the nature of the extracting solvent and environmental differences. The low

melting temperature range of 24.4°C at room temperature (25°C) is indicative of high unsaturated fatty acid constituent in the oil. The free fatty acid content and high acid values showed the crude nature of the oil and also serves as an indicator of the extent of hydrolysis by lipolytic enzymes and possibly oxidation (Gordon, 1993). The saponification value obtained in this study is high, showed that the oil contains more of low molecular weight fatty acids and is recommendable for soap making (Oladiji *et al.,* 2010).

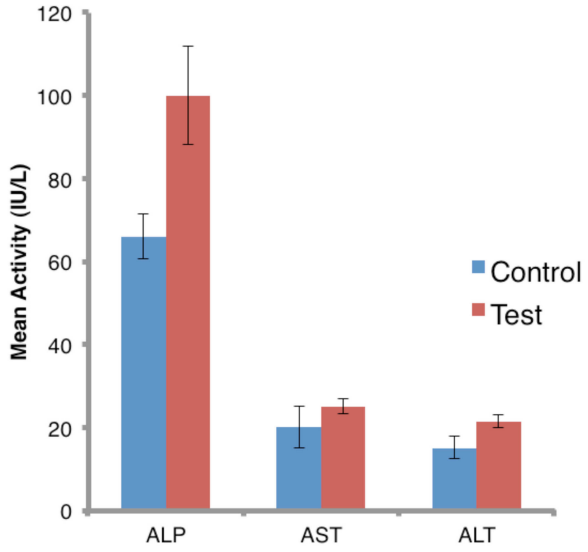

Figure 1: Effect of the oil extract of Mucuna pruriens seeds on the activities of liver function enzymes

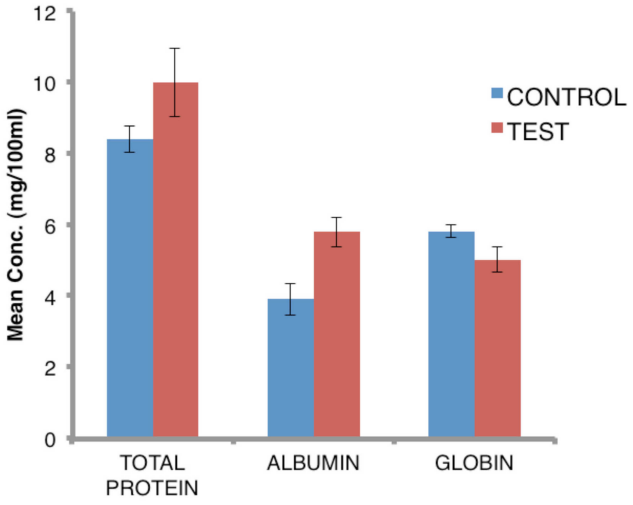

Figure 2: Effect of the oil extract of Mucuna pruriens seeds on total protein, albumin and globulin concentrations

Peroxide value is an index of resistance of oil to lipolytic hydrolysis and oxidative deterioration (Popoola and Yangomodou, 2006). The peroxide value of the oil from *M. pruriens* is in line with the range of 0-10mEq/kg stipulated for freshly prepared oil by Cooks and Reds (1966). The peroxide value obtained showed that the oil is less prone to rancidity under storage. The low density value of 0.39 showed that *M. pruriens* oil is mostly composed of unsaturated low molecular weight fatty acids. Also, the viscosity value in this study is comparable with the value reported by Njoku (1993), (40.29-41.62) for rubber seed oil, rapeseed oil 37.82, sunflower oil 34.9 and soyabean oil 32.60 (Peterson *et al.,* 1983; Strayer *et al*, 1983).

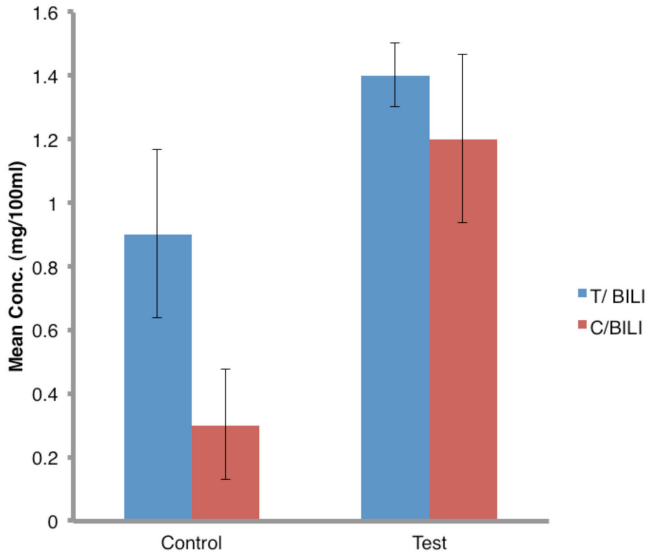

Figure 3: Effect of the oil extract of Mucuna pruriens seeds on total and conjugated bilirubin concentration

This report also confirms the low degree of intermolecular attraction of short chains fatty acids. The oil contains mostly unsaturated fatty acids (linoleic, oleic and myristoleic acids). Linoleic acid is an essential fatty acid necessary for the synthesis of prostaglandins that regulate the contraction and relaxation of smooth muscle tissue (Nelson, 2005).

Serum enzyme measurements are a valuable tool in clinical diagnosis that provides information on the effect and nature of pathological damage to any tissue. Alanine aminotransferase and aspartate aminotransferase are useful enzyme makers of liver cytolysis (Afolayan and Yakubu, 2009). Alkaline phosphatase is a marker enzyme of damage to the plasma membrane and endoplasmic reticulum (Shahjahan *et al*., 2004). It is often used to assess the integrity of the plasma membrane (Akanji *et al*., 1993). The observed increase in the activities of these enzymes in the serum in this study is attributed to damage of structural integrity of the liver, probably through leakage from the altered cell membrane structure.

Bilirubin is an important catabolic product of blood with biological and diagnostic values (Yakubu *et al*., 2005). The increase in total and conjugated bilirubin concentration in all the rats fed *Mucuna pruriens* oil based meal in this study,

maybe as a result of liver damage in which case bilirubin accumulated in the blood and possibly, the kidney was also impaired and could not filter them out of the blood. Creatinine and urea are major catabolic products of muscle and protein metabolism. The rise in serum urea and creatinine in this study might be due to impairment in renal function (Cameron and Gregar, 1998).

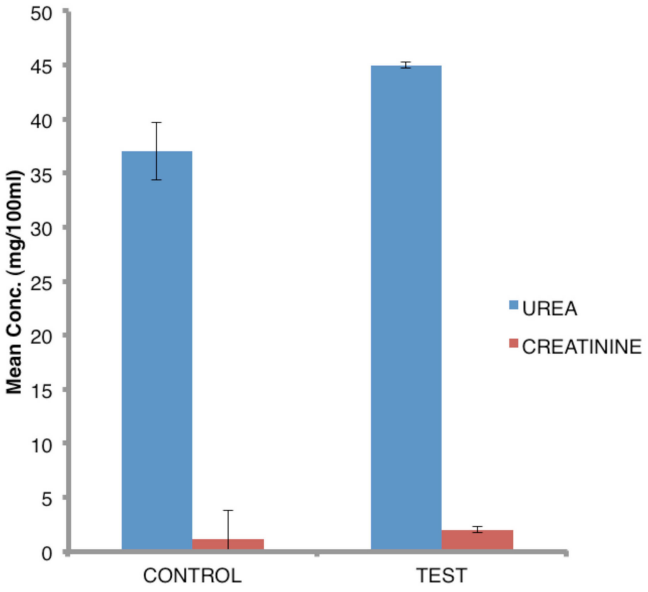

Figure 4: Effect of oil extract of Mucuna pruriens seed on urea and creatinine concentration

Serum total protein and albumin concentrations were slightly increased indicating some stimulatory effect on the protein synthesis process by certain components of the oil meal, thereby increasing enzyme activities. Alterations in liver and kidney histology observed in this study suggest that *M. pruriens* oil based diet has the potential to cause organ damage.

We conclude that rats fed *M. pruriens* oil based diet showed alteration in some liver and kidney function parameters as well as histological examinations. These findings indicate that *M. pruriens* oil based diet could be hepatotoxic and its use should be discouraged.

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