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NUTRITIONAL PROPERTY OF *MUCUNA PRURIENS* L. SEED POWDER AND EFFECT OF ITS GRADED DIETARY SUPPLEMENT ON SERUM CORTICOSTERONE IN MALE AND FEMALE ALBINO RATS

Ashidi, J. S.[1], Sonaya, O.E.[1]*, Feyisola, R.T.[1], Owagboriaye, F.O.[2], Sanusi A.S.[1] and Lawal, O.I.[1]
[1]Plant Science Department, Olabisi Onabanjo University, Ogun State, Nigeria
[2]Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ogun State, Nigeria
Corresponding Author Email: *dhammysho@gmail.com*

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ABSTRACT

Mucuna pruriens(MP) is highly recognized for its diverse biology activities. Its seeds are not only utilized in Asian countries but also here in Nigeria. Hence, the need to evaluate Mucuna pruriens seed powder for its nutritional property and influence on the level of corticosterone hormone when supplemented with rat feed at varying doses. Rats were randomized into 4 groups and fed diets supplemented with graded inclusion levels of MP seed powder for 6 weeks; group 1 (control) was maintained on normal diet while group two, three and four were fed diets supplemented with the powder of MP seed at 0.5, 1 and 1.5 g corresponding to 3.33%, 6.67% and 10% inclusion level respectively. Phytochemical, proximate, L-Dopa, minerals and heavy metal compositions were analyzed using standard methods while serum level of corticosterone in rats was analyzed with Enzyme Linked Immuno-sorbent Assay after consecutive feeding. Saponins, steroids, alkaloids, flavonoids, phenolics, triterpenes, terpenoids, coumarins and glycosides were all present in MP seed powder. Proximate analysis revealed slightly-high carbohydrate (44.70), protein (28.80) and L-Dopa (3.11g/100g) contents. Mineral analysis revealed appreciable level of calcium (125.04 ppm), magnesium (3.31ppm) and iron (2.16 ppm). Level of serum corticosterone in both male and female rats fed diet supplemented with MP at 3.33%, 6.67% and 10% compared to their respective controls was not significantly different ($p>0.05$). It is thus concludedthat MP seed powder possess nutritional potentials and may not induce stress in animal at inclusion levels of 3.33%, 6.67% and 10%.

Keywords: Corticosterone, *Mucuna* seed, Stress hormones, Velvet bean

INTRODUCTION

Stress hormones are of utmost importance in assisting the body manage and respond to stressful situations. Corticosterone is a stress-related hormone exocytosed from the cortex of adrenal gland in response to environmental stressors (Neil, 2004). In humans onset of stress is often marked with increased secretion of cortisol or corticosterone (Jacobson, 2005; De Kloet and Rinne, 2007; Franklin *et al.*, 2012). Studies have revealed that high levels of these hormones in the body can negatively impact human and animal health, if left unchecked. However, there have been reports that the seed powder of *Mucuna pruriens* relieves stress.

Mucuna pruriens or Velvet bean is in the family Fabaceae. It is also referred to as Cow witch and Cow hage with a characteristically long hairy pod (Lampariello *et al.*, 2012). The Yorubas of

Nigeria call it 'werepe'. *M. pruriens* has wide range of recorded pharmacological uses such as anti-diabetic, aphrodisiac, anti-neoplastic, anti-epileptic and anti-microbial activities (Sathiyanarayanan and Arulmozhi, 2007).

Mucuna pruriens seeds are consumed as food by humans and added to animal feed in Nigeria (ThyagaRaju *et al.,* 2017). Besides compounds of pharmacological importance, some anti-nutritional agents such as lectins and protease inhibitors are also present in *M. pruriens* (ThyagaRaju *et al.,* 2017). Out of these compounds, L-Dopa is known to be the major active compound in the seed (Lorenzetti *et al*., 1998; Gurumoorthi *et al.,* 2008). Matured seeds of *M. pruriens* contain 3.1 to 6.1% of levodopa (ThyagaRaju *et al.,* 2017). Due to the high level of this active compound, a number of studies have been conducted on the possible effects of the seed on some ailments, organs and physiological functions of the body. For instance, the influence of the seed on the immune system has been demonstrated in mice (Vermal *et al.* 2014). Moreover, our previous study has reported the aphrodisiac property of *M. pruriens* seed powder in rats (Ashidi *et al.*, 2019). To the best of our knowledge, there is little or no information on the effect of different inclusion levels of *M. pruriens* seed powder on stress hormones of albino rats. Hence, the need for this study.

MATERIALS AND METHODS

Seed collection and preparation

The MP seeds (Figure 1) were collected from Ago-Iwoye, southwest Nigeria and identification was done at Elikaf Herbarium with voucher number EH/18/19/60011 in our institution. We powderdised the seed using mortar and pestle, and analysed the powder for its nutritional compositions.

Animals

A total of 64 adult albino rats comprising thirty-two (32) male and thirty-two (32) female rats of the Wistar strain, weighing 93.81 g - 155.1 g were used for this study. The rats were housed in a well ventilated and spacious room (27^{o}C±3^{o}C). Animals were acclimatized for 10 days with water and fed *ad libitum* with rat chow purchased from Animal Care Services Konsult (Nig.) Ltd before the commencement of the experiment.

Figure 1: *Mucuna pruriens* seeds

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Study design

We randomized the rats into four groups (groups one, two, three and four). Each group contained eight male and female albino rats. The control group (group 1) was fed 15 g of the rat chow every day for 6 weeks, group 2 was fed 14.5g standard rat chow mixed with 0.5 g *M. pruriens* seed powder corresponding to 3.33% inclusion level, group 3 was fed 14 g standard chow mixed with 1 g *M. pruriens* corresponding to6.67% inclusion level and rats in group 4 were fed 13.5 g standard rat chow mixed with 1.5 g with *M. pruriens* corresponding to10% inclusion level. The level of *Mucuna pruriens* seed powder inclusion was in accordance with our previous study (Ashidi *et al.,* 2019).

Sample collection

At exactly 24 hours following the last feeding regime, blood was sampled by cardiac puncture into plain sample tubes. We centrifuged the blood at 2500 rpm for 10 min and the sera obtained were stored at -20°C for corticosterone assay.

Phytochemical screening and proximate analysis

Seeds of *Mucuna pruriens*were screened to unravel the phytochemical constituents (Table 1) as previously described (Harborne 1973; Sofowora, 1993; Trease and Evans 1989). Determination of lipid, moisture content, ash, crude protein and fibre in the seed was in accordance with the Association of Official Analytical Chemists (AOAC, 2005). We used weight difference of crude protein, moisture, lipid and ash content to determine the seed carbohydrate content. Meanwhile, mineral and heavy metals compositions of the seed were analysed according to AOAC (2005).

L-dopa determination

Level of L-dopa in the seed was determined according to Myhrrman (2002) with little modifications. Briefly, 5 mL of distilled water was added to 2 g of the seed powder in a glass culture tube. The content of the tube was thoroughly mixed and allowed to stand in boiling water for 6 minutes. We later centrifuged the tube at 3000 rpm for 2 mins and the supernatants were diluted with deionized water to make 100 mL. The mixture was allowed to pass through 0.45 mm nylon membrane and cooled to 15°C. The level of L-dopa in the extract was determined using a high performance liquid chromatography connected to an ultraviolet detector with SB-C18 colunm. The mobile phase, which consists of buffer and methanol, was run at 1 mL/min and eluted at 10mL/min for up to 20 minutes with the injection volume of 40 µL. We quantified the L-dopa in the extract from the obtained standard L-dopa curve prepared by adding 1.00mM of standard L-dopa with water.

Corticosterone hormone determination

Corticosterone level in the sera of male and female rats was determined using the corticosterone Enzyme Linked Immunosorbent Assay (ELISA) kit. The assay sensitivity and range of detection were 0.029 ng/mL and 0.05-23 ng/mL respectively.

Data analysis

The obtained data were analysed with the IBM statistical package for Social Sciences (SPSS) version 20.0 (IBM Corp 2011). Analysis of variance (ANOVA) was used for the mean values comparison and the results were presented as mean ± standard error of mean. We used Students Newman

Keuls for the Post-hoc test and level of significance was set at $p < 0.05$.

RESULTS

Phytochemical composition of the *Mucuna pruriens* seeds

Result (Table 1) of the qualitative analysis of *Mucuna pruriens* seeds showed the presence of thirteen (13) phytochemicals which include saponins, tannin, phenolics, steroids, flavonoids, triterpenes, coumarin, glycoside, anthocayanin, terpenoids, phlobatanin, amino acid and alkaloids. Steroids (227.52±0.33 mg/100g) and triterpenes (165.77±0.42 mg/100g) respectively were the highest phytochemical components of *Mucuna pruriens* seeds (Table 2). These were followed by flavonoids, phenolics, alkaloids and terpenoids respectively. However, saponins had the least concentration.

Table 1: Phytochemical screening of *Mucuna* seeds

Phytochemical composition	Result
Saponins	+
Tannins	+
Phenolics	+
Steroids	+
Flavonoids	+
Triterpenes	+
Coumarins	+
Glycosides	+
Anthocyanins	-
Terpenoids	+
Phlobatanins	-
Alkaloids	+
Amino acids	-

Key: - Absent, + Present

Table 2: Quantitative phytochemical composition (mg/100g) of *Mucuna* seeds

Phytochemical composition	Mean±SD
Saponins	0.76±0.00
Steroids	227.52±0.33
Flavonoids	56.86±0.04
Phenolics	44.57±0.04
Alkaloids	42.31±0.00
Triterpenes	165.77±0.42
Terpenoids	22.92±0.02
Coumarins (µg/100g)	7.61±0.00
Glycosides	8.71±0.02

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Proximate composition and level of L-Dopa in *Mucuna pruriens* seeds

The seedscontain some levels of moisture content, ash, carbohydrate, calorie, crude protein, crude lipids, crude fibre and L-Dopa (Table 3). Moisture content of the seed was (10.81±0.06 %). It also contained calorific value, crude protein and carbohydrate contents of 1516.65±2.32kJ /100g, 28.80±0.55 % and 44.70±0.64 % respectively.

Mineral and heavy metal compositions of *Mucuna pruriens* seeds

Results (Table4) also showed the presence of some minerals in the *Mucuna pruriens* seeds. These include zinc, phosphorus, potassium, calcium, magnesium and iron. Trace levels of selenium and lead were also recorded. Of all the mineral compositions of *Mucuna* seeds, calcium is abundantly present and higher (125.04±2.00 ppm), followed by magnesium (3.31±0.20 ppm) and iron (2.16±0.10 ppm) respectively.

Table 3: Proximate composition and level of L-Dopa in *Mucuna pruriens* seeds

Proximate composition %	Mean±SD
Moisture	10.81±0.06
Ash	4.81±0.11
Carbohydrate	44.70±0.64
Calorific value kJ/100g	1516.65±2.32
Crude protein	28.80±0.55
Crude Lipids	7.63±0.02
Crude Fibre	3.24±0.06
L-Dopa (g/100g DM)	3.11±0.03

Table 4: Minerals and heavy metals composition(ppm) of *Mucuna pruriens* seeds

Mineral composition	Mean±SD
Se	0.39±0.01
Zn	1.06±0.04
Pb	0.36±0.00
P	1.93±0.02
Ca	125.04±2.00
Mg	3.31±0.20
Fe	2.16±0.10

Level of stress hormones in the blood

Serum corticosterone observed in in the control male and female rats as well male and female rats fed with 3.33%, 6.67% and 10% of *Mucuna pruriens* seed inclusion in diet was not significantly different ($p>0.05$) (Figure 2).

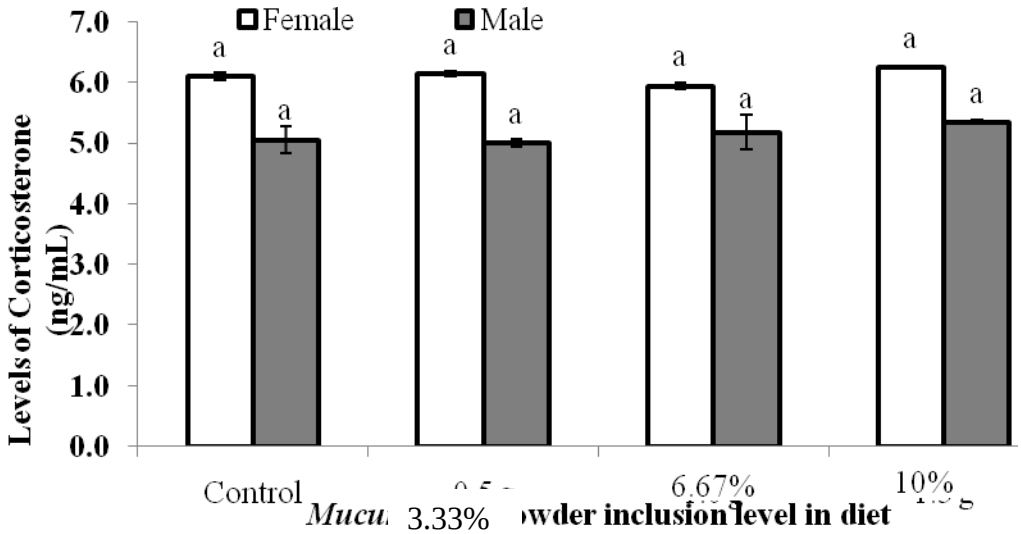

Figure 2: Serum corticosterone in male and female rats fed with varying concentrations of *Mucuna pruriens* seed.

DISCUSSION

Seeds of *Mucuna pruriens* have been globally proposed to be consumed as food by humans and supplemented with animal feed. Phytochemical analysis revealed that the seeds are rich in steroids (227.52±0.33 mg/100g), triterpenes (165.77±0.42 mg/100g) as well as phenolics, flavonoids, coumarin, glycoside, anthocyanin, terpenoids, phlobatannins, amino acids, alkaloids and saponins contributing to its notable medicinal potential. This finding is supported by Adebowale *et al.* (2005) who also noted a high level of these phytochemical constituents in *Mucuna pruriens* seeds.

From this study, seeds of *Mucuna pruriens* have high calorific value (1516.65 KJ/100g), appreciable amount of crude protein (28.80 %) and carbohydrate contents (44.70±0.64 %) including non-protein L-dopa (3.11 g/100g) of dry matter, indicating its nutritional value. This result was also confirmed by Adebowale *et al*. (2005), although, higher level of L-Dopa (4.99±0.03) was reported as against the value of 3.11±0.03 recorded for the seed used in this study.

Our results showed that calcium was the abundantly present mineral element above others. Moreover, the seeds also have high magnesium and iron contents as previously documented. For instance, Ravindran and Ravindran 1988 as well as Rajaram and Janardhanan 1991 had earlier documented the mineral content of *Mucuna utilis*: (calcium, 250.0 mg/100g) and *Mucuna gigantean*: (calcium, 518 mg/100 g). Surprisingly, lead was detected at low concentration (0.36 ppm) in *Mucuna pruriens* seeds. Although, the reason for the presence of lead in the seed is unknown, but we suspect metal pollution at the site where the seed was planted and harvested. On the contrary, Adebowale *et al.* (2005) reported lead below detection limit in the seed of *Mucuna pruriens*. Our findings therefore show that the site or location where *Mucuna pruriens* seeds were obtained should be taken into consideration in the determination and quantification of the nutritional properties of *Mucuna pruriens*.

Reports have shown an increased secretion of corticosterone as a physiological response to stress in animals (Franklin et

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al. 2012; Jacobson, 2005). It has been noted that a high serum corticosterone may induce a neurological disorder such as hippocampal dysfunction, which can result in memory function declarative deficits, reduction in neural survival, plasticity as well as cascades of promotion and inflammation (Franklin et al. 2012; Bremmer, 1999). A high level of corticosterone in the circulation may also destroy neurons by reducing glucose input and re-uptake glutamate (Sapolskey, 1995; Sapolskey, 1992). In addition, high blood pressure, muscle damage, reproductive and growth declines as well as immune suppression may also result from increased concentration of the hormone. Since the level of corticosterone was observed to be insignificantly different in male and female rats fed diet supplemented with *Mucuna pruriens* seed powder compared to their respective controls in this study, the seed at 3.33%, 6.67% and 10% inclusion level may not induce stress and the reported various physiopathological complications on the rats.

CONCLUSION

Seeds of *Mucuna pruriens* contain appreciable levels of minerals. No significant difference was observed in the serum level of corticosterone in both male and female rat's diet supplemented with *Mucuna pruriens* at 3.33%, 6.67% and 10% compared to their respective control. This study clearly shows that *M. pruriens* may possess medicinal and nutritional potential. Moreover, it may not induce stress in animal at inclusion level of 10% or less when added to diet.

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