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Molecular Characterization of a Variety of *Mucuna* and its Identification for Treatment of Parkinson's Disease

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Abstract
The genus of Mucuna consists of different species that have been reported. The sample of speciesMucunapruriens was selected and analyzed by DNA extraction, PCR amplification and Sanger Sequencing to determine its DNA sequence. The sequence obtained from the sample was compared with the sequences of other species in the NCBI database using BLAST. About 29 Sequences matched the FASTA sequence of the Sample with 95 to 98% maximum identity. The megablast was used to construct the phylogenic tree using neighbor-joining tree method to show the evolution of the sequence. The sample sequence when compared using the BLAST method showed a specific sequence which was identified as the sequence ofMucunapruriens var. pruriens. After the molecular detection and identification of the specific variety of the Mucuna, the effectiveness of its extracts on Parkinson disease was evaluated through behavioural assessment, ach estimation and histopathological studies on rats. The results obtained indicated a remarkable effectiveness of the extracts on Parkinson disease. While compared with the effect on the standard L-dopa drugs administered to the control group, the extracts were more effective on the Parkinson disease.

Keywords*: Mucunapruriens, DNA Sequencing, BLAST, PARKINSON, EXTRACT*

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I. Introduction

The genus *Mucuna*consists of 100 species of climbing vines and shrubs. Typically, *MucunaPruriens*(Velvet beans, Seabeans, Cowhage plant e.t.c), one of its species possesses stipulate leaves, always alternate and range from bipinately/palmetely compound to simple and its leaflets are 2-3 mm long. The petiole base is commonly enlarged into a pulvinus that commonly functions in orientation of the leaves. The flowers are usually bisexual actinomorphic to zygomorphic, slightly to strongly perigyrous and commonly in racemes, spikes or heads. The perianth commonly one or many stamens distinct of variously united sometimes. The pistil is simple often stipulate comprising a single style and stigma and a superior ovary with one locule containing two or many marginal ovules. The fruit is usually a legume sometimes aloments, follicle, indehiscent pod, achene, drupe or berry, ripening stage, a 4 to 13 cm long and 1 to 2 cm wide unwinged leguminous fruit develops. The seeds are Ovoid or transversely oblong slightly laterally compressed with polish dark, brown or black or occasionally mottled testa. Thickness of seed about – 0.5 mm (Divya*et al*., 2017).

Research in plant based medicine is gaining more attentions due to its positive impact on human health, especially in the area of medicinal applications. Right in the past, this has increased people's level of awareness toward functional herbal medical practices thus enhancing the use of medicinal plants in the treatment of a number of diseases (Lutharia and pastor-corrales, 2006). The use of medicinal plants species such as the species of *Mucuna*, such as *Mucunapruriens* has collosally increased since 1938, when it was initially revealed that a huge quantity of levodopa was present in some of its species

Reports on the phytochemical screening of*Mucunapruriens*revealed the presence of alkaloids, reducing sugar, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols and steroids (Minari, *et al* 2016). *M. pruriens* roots contain tangible amount bitter, thermogenic, emollient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue, anthelmintic, febrifuge, diuretic and tonic (Natarajan, Narayanan, and Ravichandran, 2012). It is considered useful in the treatment of Parkinson disease and other diseases such as

constipation, nephropathy, dysmenorrhea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, helminthiasis, fever and delirum. Specifically the *M. pruriens* seeds contains alkaloids, glycosides, reducing sugars, saponins, tannins, terpenoids, calcium, phosphorus and potassium, polyphenolic substances, protease inhibitor, phytic acid and L-dopa. Among all these extracts, L-dopa has been reported to be most effective on the treatment of Parkinson disease.

Parkinson's disease (PD) is a progressive neurodegenerative disease that leads to impaired motor function and is characterized by a loss of dopaminergic neurons in the substantianigra and is second only to Alzheimer's disease in its prevalence (Abushouk*et al*., 2017). The etiology and pathophysiologyof PD are not very well understood and have consequently stifled the development of effective therapeutic interventions for PD (Dexter and Jenner, 2013; Tapias et al., 2014). According to a Global Burden of Disease analysis, PD affected 6.1 million people globally in 2016, indicating a 2.4-fold increase in global prevalence of PD from 1990. PD prevalence increases with advancing age and peaked in the 84–89 age group in 2016. It also affects slightly more men than women, with an estimated male-to-female ratio of 1.4:1 (Dorsey *et al.*, 2018).

A slew of basic science discoveries in the mid-20thcentury eventually culminated in the trials that cemented the efficacy of L-DOPA for PD treatment (Fahn, 2015; Fahn, 2018). Dopamine was demonstrated to be present in high concentrations in the striatum of mammals (Bertler and Rosengren, 1959) and humans (Sano, 1959). Clinical study confirmed the efficacy of the M. pruriens seeds in the management of Parkinson's disease by virtue of their LDOPA content. M. pruriens has been shown to increase testosterone levels Seeds of M. pruriens contain high levels (1–6%) of L-Dopa (L-3,4-dihydroxyphenylalanine (Kavitha and Thangamani, 2014)

In a clinical study, the contribution of L-DOPA in the recovery of PD followed by Ayurveda medication. Katzenschlager et al., (2004) revealed that 30g Mucuna seed powder preparation has considerable and faster action in treating PD patients than conventional standard drugs namely, Levo-dopa or Carbi-dopa and suggested that natural source of L-DOPA might possess advantages over conventional drugs in long term management of PD.

M. pruriens consist of 4 varieties that have common morphological features, thus they are not easily differentiated. A particular specie can't easily be identified and used to serve a specific purpose especially in the area of trado-medical remedies. Therefore the purpose of the present studies was to use some molecular technique to characterize and identify a specific variety of *mucunaprurienssvar.pruriens*which has a comparatively higher medicinal properties than other varieties, especially in reference to its relatively high content of L-dopa for effective treatment of Parkinson Disease.

II. Materials And Method

COLLECTION OF PLANT MATERIAL

The seeds of Mucunapruriens were collected from some local Areas around Kaduna metropolis, Kaduna State, Nigeria. They were identified and authenticated at the Department of Biology, Nigerian Defence Academy, Kaduna.

DNA EXTRACTION

The seeds were smashed into a powder form and a solution of 95*u*l of water, 95*u*l solid tissue buffer, 10*u*l proteinase K was be added to the sample in a microcentrifuge tube. The sample was mixed thoroughly and incubated at 55^{o}c for 3 hours, after then 2 volumes of GENOMIC BINDING BUFFER was added to the supernatant and mixed. The mixture were transferred to a zymo-spin IIC-XL Column in a Collection Tube. And was centrifuged at (> 12.000xg) for 1 minute, after then the collection tube was discarded with the flow through. 400ul of DNA Pre wash buffer were added to the column in a new collection tube and centrifuged for 1 minute, after which the collection tube was emptied. 700ul of DNA WASH BUFFER were added and centrifuged for 1 minute and the collection tube was emptied afterwards. The DNA were eluted by transferring to a clean microcentrifuge tube, > 50ul DNA ELUTION BUFFER were added and incubated for 5 mins after which it was centrifuged for 1 minute.

PCR ANALYSIS

Conventional PCR were performed on genomic DNA from the proband. The AccuPowerHotStart PCR kit was used for PCR as per the manufacturer's instructions. PCR amplification was performed in the same tube as DNA synthesis by the addition of buffer, Taq polymerase, and 100pmol of each PCR primer were added (PCR amplification were performed for 45 cycles (94^{o}C, 15 sec; 50^{o}C, 45SEC; and 72^{o}C, 1 min) with a final elongation step of 10 min at 70^{o}C on a model 2400 Perkin Elmer thermal cycler. The product generated by PCR were purified and quantified before its use as a template in sequencing reactions. The PCR products were evaporated to a volume of 10-15*u*l and electrophoresed on a 1% agarose gel. PCR products were cut from the ethidium bromide-stained gel, and the DNA were extracted with the QIAquickl Gel Extraction kit as per the

manufacturer's instructions. The DNA was quantified by comparison with a 5-400-ng DNA mass ladder on an ethidium bromide-stained 1% agarose gel.

SANGER SEQUENCING

Sequencing reactions were performed with the ABI prism BigDye Terminator Cycle sequencing ready reaction kit with Amplitaq DNA Polymerase, as described by the manufacturer. The quantity of template used for sequencing was 3*u*l of 60ng DNA, 3*u*l of Tag DNA polymerase and 2.5*u*l of DEPC water. Extension products were purified with CentriSep spin columns as described by the manufacturer. Sequencing reactions were run on an ABI prism 377 Automated Sequencing apparatus.

IDENTIFICATION OF SEQUENCING PRODUCT

Basic Local Alignment Search Tool (BLAST) queries was used to compare COI sequences from the sample to all the publicly available sequences that make up the nucleotide database maintained by the National Center for Biotechnology (Altschul*et al*. 1997). From the BLAST search we retained five matches and its associated GenBank accession numbers, the total score for the top five matches as calculated by the BLAST algorithm, the query coverage in terms of the length of alignment between found and query sequence in terms of percentage of query sequence length, and maximum identity (the percent similarity between the query and found sequence over the length of their alignment).Phylogenic tree was generated from the resulting alignment by neighbor-joining (Saitou &Nei 1987)

DETECTION/EXTRACTION OF L-DOPA

The method of (Vachhani*et al*, 2011) was used for the extraction. The seed powder will be suspended in Water: Methanol (50:50) (v/v) and let it stand for 2 hours.

INDUCTION OF PARKINSONISM BY RETONONE

Adult male rats were subcutaneously injected once every 48hrs at (1.5mg/ml) of retonone dissolved in 50x stock solution of DMSO for 28 days (Jason *et al* 2010). All the behavioural assessment was carried out and observation on their behavioural patterns was made.

GRIP STRENGHT

All animals were evaluated for grip strength by using the rotatory stool. The rotatory stool test is used in rodents to assess their minimal neurological deficit such as motor function and coordination. The animals will be placed on the rotatory stool and made to grip it. This was carried out for each rat in 2 mins. The experiment was carried out on Day 0, 7, 14 following retonone administration. This activity was used to measure their muscular strength, how long does it take to hold. The average results were recorded as fall of time. (Jitendra*et al*, 2016)

LADDER WALK

The horizontal ladder test is a motor and coordination test for evaluating skilled walking for mice and rats. The rats were placed in a horizontal ladder and made to walk through it, the number of steps missed was counted, each rat were made to walk through. The average result was recorded as number of missed steps. This test will be performed in Days 1 7, 14 (Jitendra*et al*, 2016)

STEP DOWN INHIBITOR (LEARNING AND MEMORY)

The rats were evaluated for learning and memory using the inclined plane. The animals were placed in a wired electric container with a compartment that was not wired, the number of times the animal steps down from the wired compartment after first shock were recorded. This was performed for two days, on the second day the animal were placed in the inclined plane and were evaluated for remembering the activity of day 1 and restrained from touching down the wired compartment. This was done in the 4th week. (Gilvan*et al*, 2015)

DISSECTION AND HOMOGENIZATION

After the treatment period, animals were sacrificed by decapitation under mild anesthesia. The brains were immediately removed, forebrain were dissected out. Brains were put on ice and rinsed in ice-cold isotonic saline to remove blood. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 Rpm for 15 minutes and aliquots of supernatant obtained were used for Ach estimation (Jitendra*et al*, 2016)

HISTOPATHOLOGICAL STUDIES

The brains from control and experimental group were separated from the animals and fixed in 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5*u*m thickness. The sections were stained with hemotoxylin and eosin dye for histopathological observation through the use of microscope. (Jitendra*et al*, 2016)

ACH ASSAY

Biolabs Acetylcholine Assay Kits was used; 250 mg of wet tissues or cell pellet samples were homogenized in 4.5mL of chloroform/methanol at the ratio 2:1. The samples were centrifuged to remove debris. The homogenates were incubated at room temperature for an hour placed upon an orbital shaker. Added to a 96-well microtitter plate was a 50 uL of diluted acetylcholine standards as well as 50 *u*L of prepared acetylcholine reaction reagent added to each standardand samples and it was thoughroughly mixed. To prevent the penetration of light, the plate wells were properly covered and were incubated on an orbital rotator at room temperature. A spectrophotometric micro plate reader set in the 540-570 range was used to read the plate. The final results were gotten by comparing the absorbance with the standard curve of acetylcholine (Joshua *et al*, 2016)

DATA ANALYSIS

The values were expressed as mean SEM. Statistical evaluation of the data was done by one-way ANOVA (between control/Extracts) followed by Turkey's t-test for multiple comparisons, with the level of significance chosen at $p < 0.05$. The statistical product used was Graph pad prism 5 Software.

III. Results And Discussions

MOLECULAR STUDIES

From the molecular studies 100 bp Plus DNA Ladder was used in identifying the base pair region of specific variety*mucunapruriens* species, up to 574 base pair region was obtained from the sample, this is in contrast with reports of K.V Deepak *et al*(2018) which showed355 base pair region an indication that the seeds used was for a different variety.

Based on whole genome shotgun sequence, the variety of the species was identified as *MucunaPruriens* as shown in (Plate 1). Sequence from *Mucunapruriens* seed sample were obtained using PCR amplification and Sanger Sequencing. BLAST results from the samples indicated that they were *mucunapruriens var. pruriens* (Table 1). The sample matched *mucunapruriens var. pruriens* sequences in the database with 95% to 97% identity (Table 1) and this is in conformity with the report of K.V Deepak and K.V Rashmi(2018) were they identified a variety of *mucnapruriens* specie to be *mucunapruriens var. pruriens*. Phylogenetic inference placed the unknown sample as representing *mucunapruriens var. pruriens* (Figure 3)

PCR Amplification of genomic DNA in multiple individuals is a means to generate homologous DNA fragments that are end-sequenced and compared to reveal particular sequence variations. Sanger sequencing is still one of the best methods in terms of generating high quality sequencing information as many whole genome sequencing drafts were achieved using that method.(Stepahne*et al*, 2012)

Also Haynes *et al*, (2013) obtained similar result while carrying out DNA sequencing using sanger method to identify an organism by using Basic Local Alignment Search Tool (BLAST) queries to compare sequences.

In this study, BLAST queries were used to compare sequences from NCBI database to be able to identify our sample. 29 Sequences corresponded with our FASTA Sequence, with 95% to 98% maximum identity. This led to the reliable identification of the variety of *mucunapruriens*.

ACETYLCHOLINESTERASE ASSAY

The result from Figure 2 shows that the mean ACH value for group 1, 2, 3, 4, 5, and 6 are 2.462, 2.576, 1.098, 2.926, 2.092, and 3.428 g/l respectively, whereas ACH values of the control group induced with PD (group 3) is lower than ACH values of group 4, 5, and 6. Also, the analysis of variance (ANOVA) test indicates that the ACH value of group 3 is statistically significant from ACH values of group 4 and group 6 with a P-value of 0.0008 (i.e. values are assumed not to be equal) since the p-value is less than 0.05.

GRIP STRENGHT

The result from Table 2 shows that the mean grip strength on day 0 ranges from 21.6 – 41.4, on day 7 ranges from 19.2 – 37.2, and at day 14 ranges from 17.0 – 34.0. The study also showed that at day 0, 500mg MP extract (group 6) had the highest grip strength, followed by 250mg MP extract (group 5), and standard drug (group 4), with grip strength values of 41.6, 37.6, and 28.8 respectively compared to the control (group 3) with a grip strength of 21.6 which were all induced with PD. Also, at day 7, 500mg MP extract (group 6) had the highest grip strength, followed by standard drug (group 4), and 250mg MP extract (group 5), with grip strength values of 28.4, 27.0, and 22.2 respectively compared to the control (group 3) with a grip strength of 19.0. At day 14, the standard drug (group 4) had the highest grip strength, followed by 500mg MP extract (group 6), and 250mg MP extract (group with grip strength values of 34.0, 26.4, and 20.0 respectively compared to the control (group 3) with a grip strength of 17.0. The analysis of variance (ANOVA) test indicates that the grip strength values from day 0 – 14 of the control and experimental group are not statistically significant (i.e. values are assumed to be equal) since the p-values are greater than 0.05. This result is in conformity with the reports by

(Singh *et al*, 2016) where he found a significant increase in locomotor activities with *Mucunaprurien*streated animals.

LADDER WALK EVALUATION

The result from Table 3 shows that the mean ladder walk value at day 0 ranges from 10.2 – 17.8, at day 7 ranges from 10.0 – 18.2, and at day 14 ranges from 14.4 – 20.6. At day 0, none of the ladder walk value was greater than the control group induced with PD (group 3). However, at day 7, compared to the control group (group 3), ladder walk of group 4 (standard drug) and group 5 (250mg MP extract) improved, followed by group 6 (500mg MP extract) with mean ladder walk values of 18.2, 18.2, and 17.4 respectively, and at day 14, compared to the control drug (group 3), ladder walk of group 6 (500mg MP extract) improved, followed by group 5 (250mg MP extract), and standard drug (group 4), with mean ladder walk values of 20.6, 19.0, and 17.8 respectively.The analysis of variance (ANOVA) test indicates that the ladder walk value from day 0 – 14 of the control and experimental group are statistically significant (i.e. values are assumed not to be equal) since the p-values are less than 0.05.

LEARNING AND MEMORY VALUE

The result from Table 4 shows that the learning value of group 1, 2, 3, 4,5 and 6 are 0.2, 0.2, 1.6, 0.4,0.4, and 0.4 respectively. Also, PD induced mice (group 3) values were higher, while after treatment with standard drug (group 4) and MP extracts (group 5 and 6), the learning values reduced. The analysis of variance (ANOVA) test indicates that the learning values of the control and experimental group are not statistically significant (i.e. values are assumed to be equal) since the p-values are greater than 0.05.

The result from Table 5 shows that the memory value of group 1, 2, 3, 4,5 and 6 are 1.0. 1.2, 1.2, 0.4, 0.6, and 1.4 respectively. Also, PD induced mice (group 3) memory values were higher that those treated with standard drugs (group 4), and 250mg MP extract, while 500mg MP extract memory value was higher than PD induced mice (group 3) memory value. The analysis of variance (ANOVA) test indicates that the learning values of the control and experimental group are not statistically significant (i.e. values are assumed to be equal) since the p-values are greater than 0.05.

ACETYLCHOLINE

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurons of the SNpc. The pathogenesis of PD includes oxidative stress, protein accumulation like a-synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitoxicity. Acetylcholine is a type of chemical messenger, or neurotransmitterthat plays a vital role in the central and peripheral nervous system. It is important for muscle control, autonomic body functions, and in learning, memory, and attention.

The oxidative stress parameters were assessed based on the, Acetycholinesterase (ACH) Level, from the results ACH was significant.. result from the findings also showed that the ACH of standard drug (group 4) and 500 mg MP extract (group 6) is significantly higher that ACH value of PD induced mice (group 3) with a mean difference of 1.828g/l and 2.330 g/l respectively, , which are indications of antioxidant potential of MP in reducing PD. From this study we can support that *mucunaprurien*sincreases the level of acetylcholine in the brain and is therefore potent in the management of Parkinson's disease. The outcome of these findings is also in agreement with the study of (Srivastava, Naseer, and Gupta, 2022; Olabanji*et al*., 2013; Agbafor and Nwachukwu, 2011).

PARKINSONISM AND BEHAVIOURAL ASSESMENT

The behavioral assessment was investigated in terms of the grip strength, ladder walking, learning and memory, and results from the findings, the trend of the grip strength shows that*mucunaprurien*streated group performed better compared to the control group induced with PD without treatment (group 3),At Day 0 the grip strength of 500mg MP (group 6) extract was the highest, followed by 250mg MP extract (group 5) and standard drug (group 4) with mean differences of 6.4, 7.6, and 5.0 respectively at day 0, while at day 7, the grip strength of 500mg MP (group 6) extract was also the highest, followed by standard drug (group 4), and 250mg MP extract (group 5) with mean differences of 8.0, 8.2, and 7.4 respectively, while at day 14, the grip strength of standard drug (group 4) was the highest a, followed by 500mg MP (group 6) extract and 250mg MP extract (group 5) with mean differences of 3.4, 4.6, and 6.2 respectively. Although these values were not significant, it shows that the grip strength increases from day 0-14 upon administering standard drug and MP extracts which is an indication that the MP extracts have the ability to increate spontaneous locomotor activity,From The result of the grip strength, on Day 7 we can see that the extract groups performed better when compared to the standard drug, even though the values of the standard drug was higher in day 14, but this shows the effectiveness of the Extract in improving motor coordination and muscle strength.which is also in agreement with the findings of (Singh *et al*., 2016).

For the ladder walking assessment, Number of steps missed was calculated. Parkinson disease reduces cognitive behavior and therefore makes walking difficult. Cognitive Behaviour of the Group 3 induced rats reduced when compared to the number of steps missed, it did not miss much steps due to the reduction in motor movements. results from the findings indicates that at day 0, ladder walk of group 4 and 5 did not miss much steps compared to group 3 with mean difference of 6.4 and 7.6 respectively which is due to the fact that treatment just commenced, at day 7, number of steps missed of group 4, 5, and 6 significantly improved compared to group 3 with mean differences of 8.0, 8.2, and 7.4 respectively, and at day 14, number of steps missed of the extracts (group 4, and 5) significantly improved compared to group 3 with mean differences of 4.6, and 6.2 respectively. The outcome of these findings shows that after PD inducement, number of steps missed at day 7 and 14 was significantly improved after administering standard drugs, and MP extracts. However, at day 7, the 250mg extract had the highest number of steps missed, and at day 14, the 500mg extract had the highest number of steps missed. Hence, the MP extracts significantly improves locomotor activity. From the Result of the ladder walk we can see that the extract performed excellently well, even though the values for the Standard drug and Extract are the same, this shows that the extracts matches the ability of the standard drug in improving memory and coordination in wistar rats. This conforms with the reports of (Debora &Ole, 2022)

In terms of the learning and memory behavioral assessment, results from these findings indicates that learning reduces after PD inducement since both the standard drug and MP extracts treatment couldn't improve learning value compared to the PD induced control group (group 3) which in contrast to the findings of (Sachan et al., 2015; Poornachandra et al., 2005). However, memory also reduced after PD inducement, but increased after treatment with 500mg MP extract (group 6). The outcome of these findings shows that 500mg MP extract improves memory enhancement compared to standard drugs, and although cognitive enhancer activity of *Mucunapruriens* was comparable to that of standard drugs, it failed to improve learning of the mice. This however indicates that PD induced mice predate motor symptoms (Jankovic and Tan, 2020), due to degeneration of neurons in the pars compactasubstantianigra, causing dopamine depletion in the striatum (DeLong and Wichmann, 2007).Parkinson's disease is known to significantly reduce motor, locomotor activities, with also some effects on memory. This study investigated the effect of the treatment with *mucunapruriens* in improving motor and locomotor activities, from this study we can see that the group administered 500mg of extracts showed the highest increase in this activity when compared to the other groups.

HISTOPATHOLOGY

As seen in Plate 2Histopathological findings showed that methanolic extract of *mucunapruriens*treated animals had decreased infiltration of neutrophils, reduced intracellular space, increased density of cells and regained normal architecture Slight moderate necrosis in stratium region of the brain. This is in line with the research done by (Jitendra 0. Bhangale and Sanjeev R. Acharya, 2016) we can see from plate 2 that the groups treated with mp recovered only showing slight necrosis with group 6 showing normal feature, which is an indication on the ameliorative effects of *mucunapruriens* on Parkinson's disease.

This study has shown and proven that *mucunapruriens*has significant effect in the treatment of Parkinson disease. High dose of *mucunapruriens* in this study has shown greater effect in treating Parkinson disease even when compared to the standard drug. This is an indication to the recommendation of adopting herbal treatment in the treatment of this disease. The basis underlying the success of using L-dopa in specific variety of mucuna for effective treatment of Parkinson disease is the reliable identification of the variety through molecular characterization that is accountable for its peculiar morphological features that are not easily distinguished from other varieties through physical observation in their morphology.

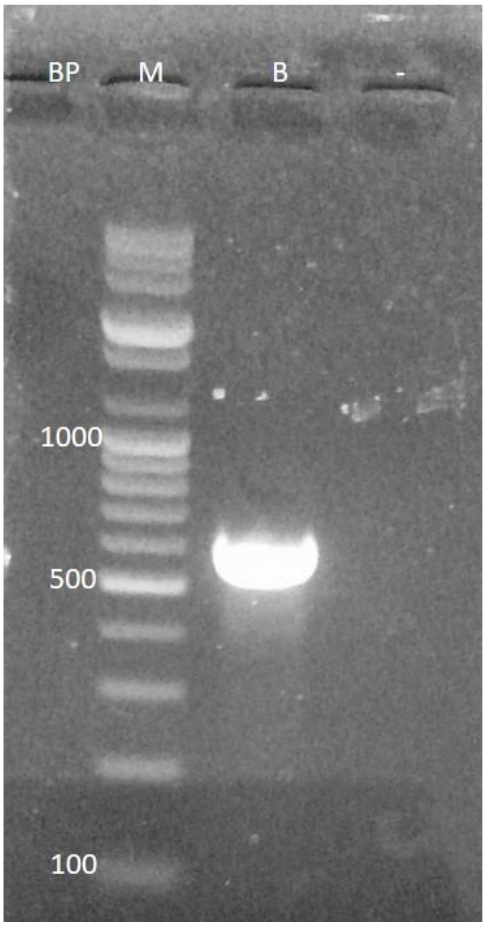


Figure 1: Agarose gel analysis of target organisms after PCR amplification of DNA (M) Molecular Marker of 100bp; (2) *Mucunaprurien* showing 547-bp

Primer Sequence: Rbcla F ATGTCACCACAAACAGAGACTAAAGC

Rbcla R GTAAAATCAAGTCCACCRCG

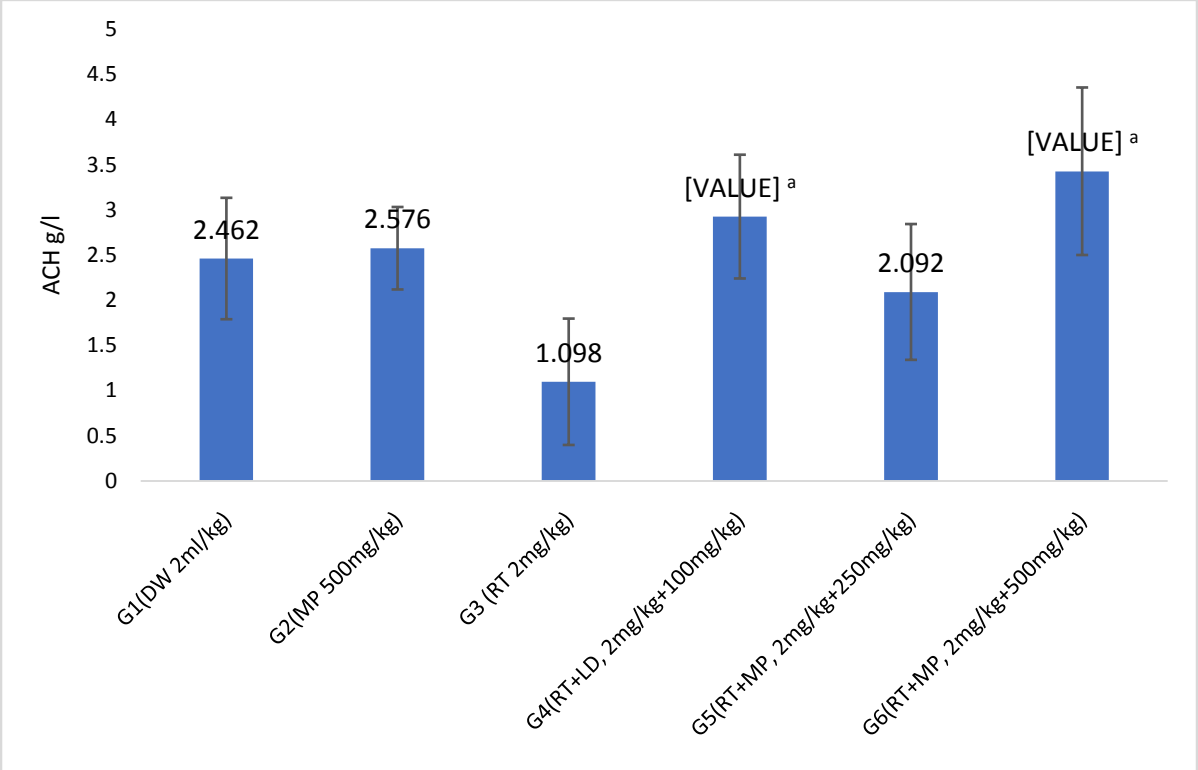


Figure 2: Acetycholinesterase (ACH) Level

Note: a = values with superscript 'a' are significant from group 3
b = values with superscript 'b' are significant from group 1

TABLE 1
Summary of megablast queries of the National Center for Biotechnology Information nucleotide collection database

Query	Matching variety	Rank	Total Score	Query Coverage %	Maximum identity, %	Accession number
Unknown Sample: 574bp	Mucunaprurien var. prurien	1	883	99	95.88	LC494390.1
	Mucunaprurien var. prurien					
	Mucunaprurien var. prurien	2	883	99	95.88	MG946853.1
	Mucunaprurien var. prurien					
	Mucunaprurien var. prurien	3	883	99	95.88	KX119311.1
	Mucunaprurien var. prurien	4	878	99	95.70	EU128734.1
		5	872	96	96.47	MH549925.1

Table 2: Grip Strength

Categories	DAY 0	DAY 7	DAY 14
GROUP 1	31.8±17.87	27.4±3.05	20.4±12.03
GROUP 2	41.6±45.64	37.2±9.985	21.2±11.34
GROUP 3	21.6±9.788	19±2.000	17±2.55
GROUP 4	28.8±9.654	27±10.93	34±9.083
GROUP 5	37.6±19.46	22.2±6.017	20±4.583
GROUP 6	41.6±18.17	28.4±12.82	26.4±9.813
P-value	0.7216	0.0565	0.0723
Df	5	5	5
F	0.5708	2.676	2.343
Remark	ns	ns	Ns

Table 3: Ladder Walk

Categories	DAY 0	DAY 7	DAY 14
GROUP 1	17.4±5.55	15.6±3.847	19.6±2.074
GROUP 2	13.0±1.581	16.2±1.483	20.4±2.302
GROUP 3	17.8±2.049	10.0±1.225 [b]	14.4±1.342 [b]
GROUP 4	11.4±1.673 [a]	18.0±2.449 [a]	17.8±1.789
GROUP 5	10.2±3.834 [a,b]	18.2±1.095 [a]	19.0±200 [a]
GROUP 6	12.8±2.588	17.4±1.673 [a]	20.6±1.14 [a]
P-value	0.0035	0.000	0.000
Df	5	5	5
F	4.795	9.882	8.042
Remark	s	s	S

Note: ns = not statistically significant
s = statistically significant
a = values with superscript 'a' are significant from group 3
b = values with superscript 'b' are significant from group 1

Table 4: Learning

Categories	Learning	P-value	F	df	Remark
GROUP 1	0.2±0.4472	0.143	2.735	5	Not significant
GROUP 2	0.2±0.4472				
GROUP 3	1.6±1.14				
GROUP 4	0.4±0.8944				
GROUP 5	0.4±0.5477				
GROUP 6	0.4±0.5477				

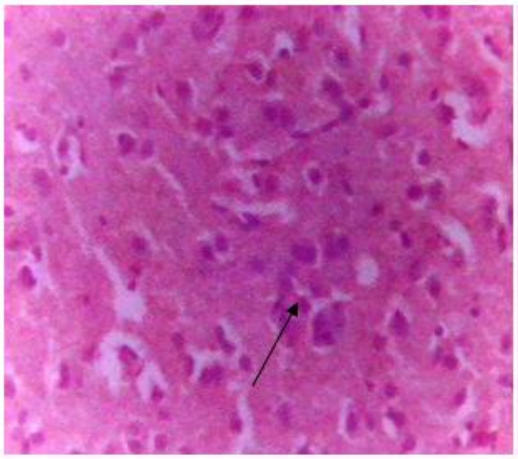
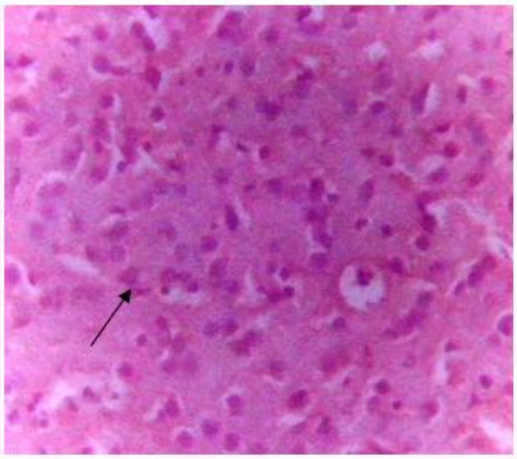
Table 5: Memory

Categories	Learning	P-value	F	df	Remark
GROUP 1	1.0±1.000	0.1206	1.965	5	Not significant
GROUP 2	1.2±0.4472				
GROUP 3	1.2±0.4472				
GROUP 4	0.4±0.5477				
GROUP 5	0.6±0.5477				
GROUP 6	1.4±0.5477				

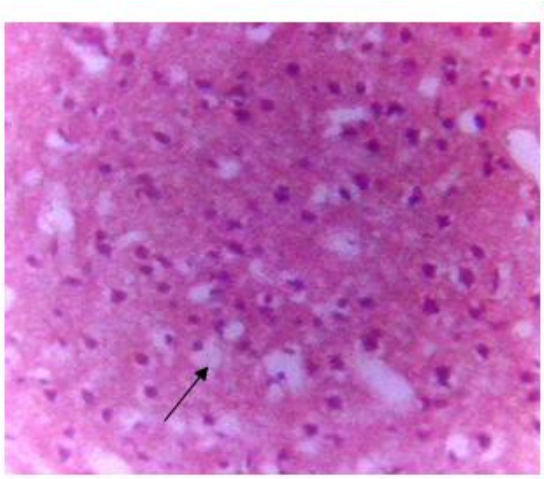
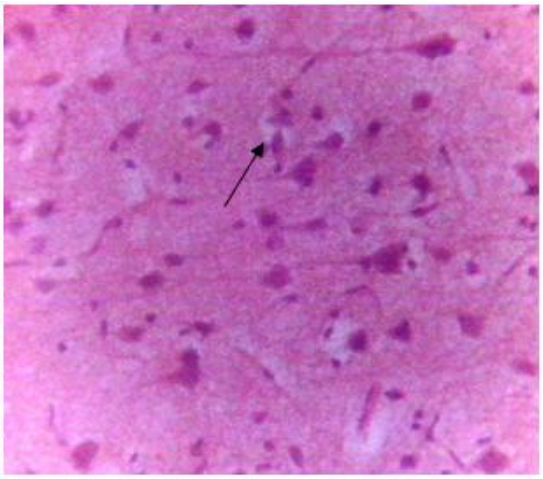
Fig 3. Neigbour-joining tree of Mucunaprurien sequences generated from NCBI MEGABLAST. The tree contains 29 sequences, 28 from GenBank and one determined for this study.

PLATE 1: A variety of *Mucunapruriens*

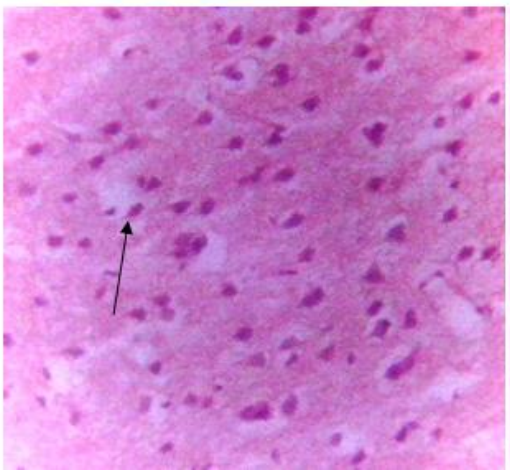
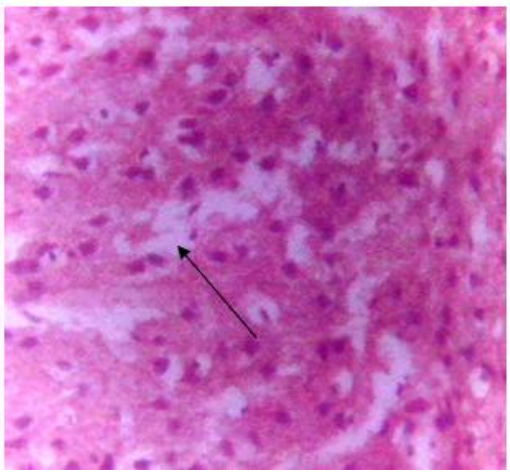
PLATE 2: Histopathological findings

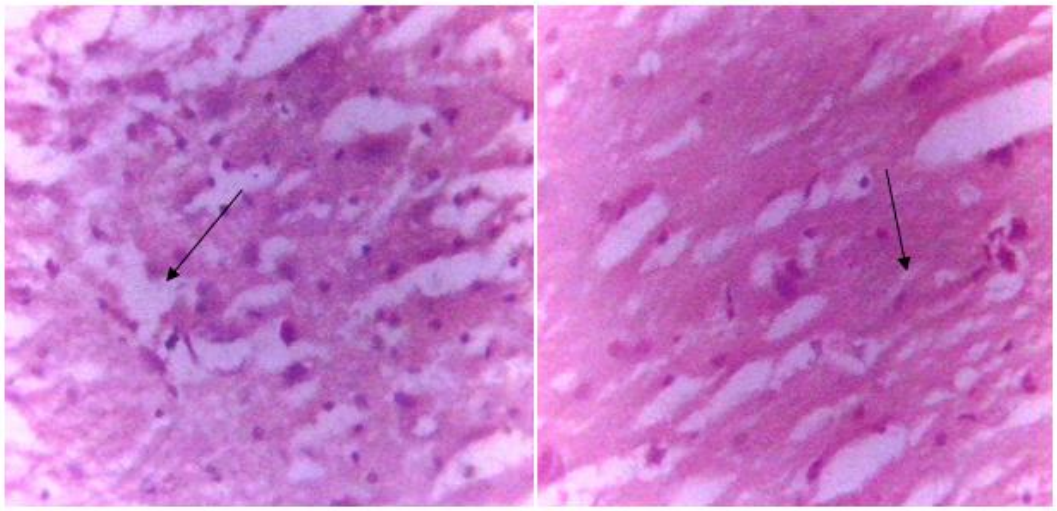
GP 1 NORMAL NEURONES GP 1 NORMAL NEURONES

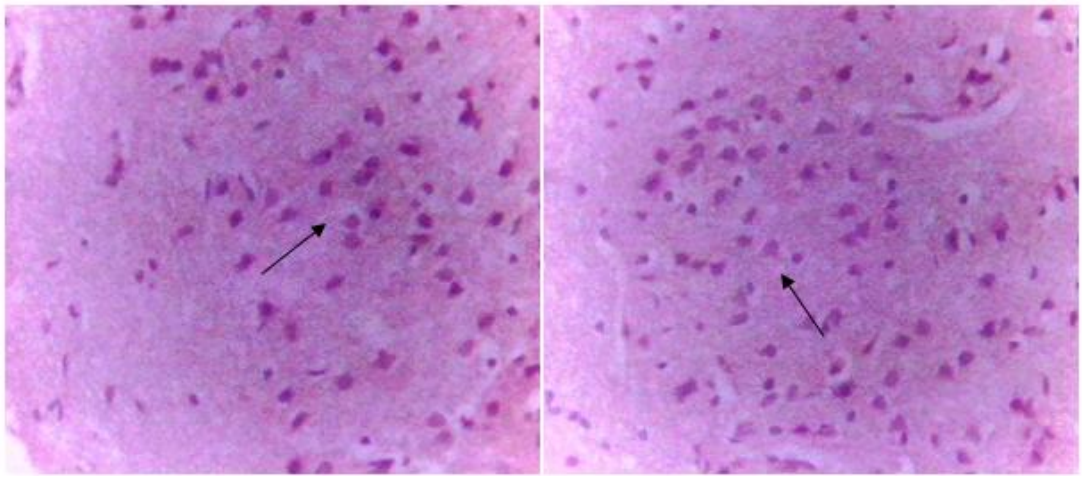
GP2 MODERATE NEURONAL NECROSIS GP2 SLIGHT NEURONAL NECROSIS

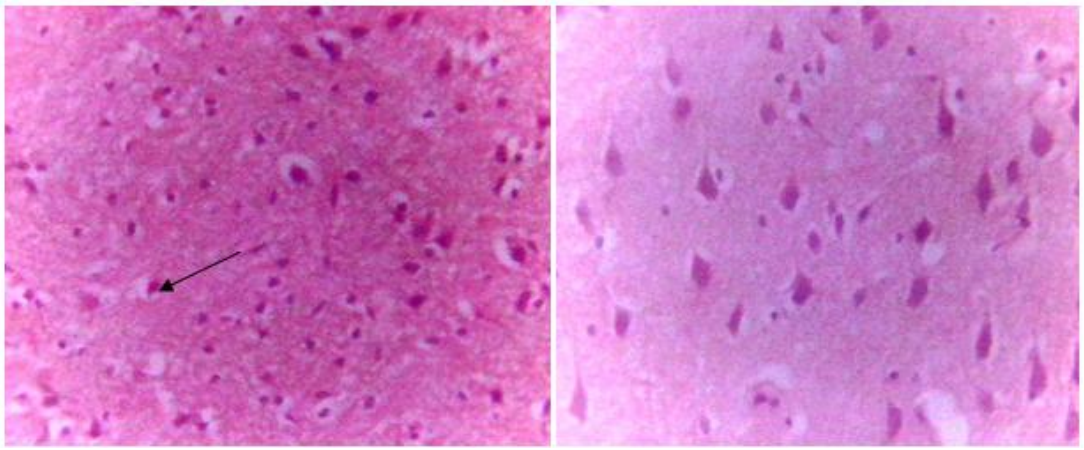
GP3 SLIGHT NEURONAL NECROSIS GP3 MODERATE NEURONAL NECROSIS



GP4 MODERATE NEURONAL NECROSIS GP4 MODERATE NEURONAL NECROSIS



GP 5 SLIGHT NEURONAL NECROSIS GP5 SLIGHT NEURONAL NECROSIS



GP 6 SLIGHT NEURONAL NECROSIS GP6 NORMALFEATURE

CONFLICT OF INTEREST

There is no conflict of interest for this study.

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