Original Research Article

Molecular analysis of sedative potential of PREP1 gene in *Mangifera Indica* flower as a remedy for insomnia.

ABSTRACT

Aims: The study was aimed at the molecular characterization of the flower of *Mangifera indica* for the presence of Pre-sequence Protease 1 gene.
Place and Duration of Study: Sample: The fresh flower of *Mangifera indica* (Mango) were collected using secateures and taken to the Department of Biological Sciences, Nigerian Defense Academy, Kaduna for specific identification and authentication.
Methodology: *AccuPrep* Genomic RNA Extraction Kit (K-3032) was used for the extraction of the RNA from the flower of *Mangifera indica*. Molecular weight of the marker gene was used in indicating a high presence of PREP1 gene in *Mangifera indica* flower**.**
Results: A high presence of PREP1 gene in *Mangifera indica* flower was detected using the molecular weight of the amplified bands.
Conclusion: A high presence of PREP1 gene in *Mangifera indica* flower is a sign of the possibility that the plant sample can feature in the future as a better remedy for treatment of insomnia

Keywords: Insomnia, Sedatives, PREP1 gene, Mangifera indica Flower.

1. INTRODUCTION

Cases of insomnia and other sleep disorders have increased considerably over the years and have become prevalent diseases affecting a high percentage of the world population, some of these sleep disorders are sleep apnea, leg cramps, narcolepsy, jet legs, restless legs syndrome, sleepwalking, insomnia [5].

Sleep Disorders (SDs) are particularly increasing in students as they face multiple stressors such as academic overload, constant pressure to succeed, and concerns about the future that alter the quality of their sleep. Socio-demographic factors such as age and gender, sleeping hygiene, physical illnesses and mental disorders were identified to correlate with insomnia as well as individuals with family histories of depression or anxiety and who manifest life-long depression and anxiety beginning in childhood are at uniquely high risk for insomnia at midlife [8]. It is the primary risk factor for depression [3] and contributes to risks of cardiovascular disease [16], type 2 diabetes [6] and obesity [10].

PREP1 overexpression is known to inhibit MEIS1 tumorigenesis according to [12]. MEIS1 contains genes that activate pathways promoting tumor growth likewise insomnia while PREP1 targets genes from the same categories, but acting as inhibitors of those pathways. With this it can be proposed that the expression of PREP1 gene will inhibit the overexpression of MEIS1 an insomnia related gene.

Over the years, various parts of *Mangifera indica* have been known to possess antioxidant, hypolipidemic, anti-inflammatory, analgesia mediated by opioid receptor affinity, sympathomimetic, endocrine, antimicrobial, antiparasitic, sedative, hypotensive, properties, soothing cure for rheumatism and toothache [1]. The previous use of the plant parts as sedatives in the treatment of insomnia [14], [19] and

other related illnesses is a convincing factor that the gene of interest (PREP1 gene) might be present in the plant. Numerous phytochemical and proximate content of *Mangifera indica* do not just clarify its edibility but its therapeutic effects as well with little or no risk of addiction due long usage as observed in conventional drugs. Limited evidence based option for treatment of insomnia, increasing number of people being affected by insomnia and the availability, accessibility and affordability of these plants are important factors that will be of great advantage in their use as remedy for insomnia.

This study was conducted to meet the need to identify and molecularly characterized the presence of PREP1 gene and as well assay the proteins in the plant samples for the presence of some essential amino acids such as tryptophan among others which the body uses for synthesis of several important molecules such as serotonin and melatonin hormones that regulate sleep-wake cycle.

2. MATERIAL AND METHODS

2.1 *Cell disruption/ lysis*

To extract the cells of the plant samples, vigorous cell lysis via grinding method was done as follows: The the fresh flowers of mango (*Mangifera indica*) were washed with distilled water and then cleaned with tissue paper to remove moisture, cut with scissor and grounded using a mortar and pestle that has been pretreatedcx with 100% ethanol before conducting RNA and DNA extraction using the following method;

2.2 *RNA extraction Basic Protocol*

The *AccuPrep* Universal RNA Extraction Kit (K-3140, K-3141) was used. But before the extraction;

- 10µl of β-mercaptoethanol was added per 1ml RB buffer.
- Additional ethanol (80% and 100%) was prepared and kept separately.

i. 500µl of RB Buffer was added to a maximum of 100mg tissue (lysed plant sample) and vortex vigorously.
ii. Sample was incubated at 60℃ for 5min.
iii. Then centrifuged at full speed for 2min.
iv. The supernatant, aqueous phase was transferred to a new microcentrifuge tube and 0.5 volume of 90%ethanol was added and mixed immediately using a pipette.
v. The sample was transferred to a binding column in a 2ml collection tube and centrifuged at 14,000 rpm for 20sec.
vi. The flow through from the collection tube was discarded.
vii. 700 µl of RWA1 buffer was added carefully without wetting the rim and centrifuged at 14,000 rpm for 20sec.
viii. The solution from the collection tube was discarded then 500 µl of RWA2 Buffer was added without wetting the rim and centrifuged at 14,000rpm for 20sec.
ix. The solution from the collection tube was discarded then 500 µl of RWA2 Buffer was added without wetting the rim and centrifuged at 14,000rpm for 2min.
x. The solution from the collection tube was discarded and centrifuged one more time at 14,000 rpm for 1min to completely remove ethanol, and properly checked for any traces of droplet clinging to the bottom of the binding column tube.
xi. The binding column tube was transferred to a new 1.5ml tube for elution.
xii. 200 µl ER Buffer was added to the binding column and kept for 2min at room temperature then centrifuged at 10,000 rpm for 1min to elute and kept in a freezer till the time of usage.

Constitute Primer

For each 100stock of primer, 100 µl of double distilled water was added.

Prep-1 Forward primer Prp-1F (5'TCCCCAGTCGCAATCTTCGC3')

Reverse primer Prp-1R (5'AAGTGGAGCGGGTTGTGTGG3')

Prep-1 Forward primer Prp-2F (5'TCCCCAGTCGCAATCTTCGC3')

Reverse primer Prp-2R (5'AGAGGCGAGAGAACTGCCCA3')

2.3 *cDNA reverse transcription*

To synthesize the cDNA, 2 µl of primer, 16 µl of total RNA and 2 µl of water was added to a 2 step RT premix, and run in a PCR machine at 42℃ for 1hr and 95℃ for 5min. However, this is only applicable to RNA.

2.4 *Run amplicon (Conventional PCR)*

For the conventional, 2 µl of primer, 16 µl of double distilled water and 2 µl cDNA/DNA product as the case may be were added into a Hot start premix and run at 52℃ and 49℃ annealing temperatures.

2.5 *Agarose gel electrophoresis*

To prepare the gel, 1.8% agarose was added to 100 µl 1xTAE buffer and 14 µl dye-Etidium bromide was added and kept in an oven to properly dissolve agarose before being kept to cool at room temperature and poured into the gel tank already containing the gel comb to finally solidify at room temperature overnight. To load the gel, 7 µl of PCR product was loaded into each well of the gel along-side a 100kb DNA ladder and allowed to run for 35min. The gel was visualized under a UV spectrophotometer.

2.6 *Sequence determination of the amplified gene*

The amplified gene(s) of the sample was processed for sequencing and characterization. The sequencing kit (Applied Biosystems) with the product was analyzed with ABI prism DNA sequence (ABI). The gene sequence of each plant sample obtained in this study was compared with known proteins/gene sequences in the Gene Bank database as described by [17].

3. RESULTS AND DISCUSSION

Results obtained on the gel image below in figure 1.5 showed visible bands at the exact region of the molecular weight of the primers used. The gel is labelled as thus; the first well was empty while the second was loaded with the 100bp molecular weight marker (DNA ladder). The third, fourth and fifth well of the gel were all loaded with the PCR product plant sample Mangifera indica flower. The label 'a' is pointing to the DNA band on the three wells of the gel containing the sample at 226 bp while 'b' is pointing to the band at 163bp of the DNA ladder.

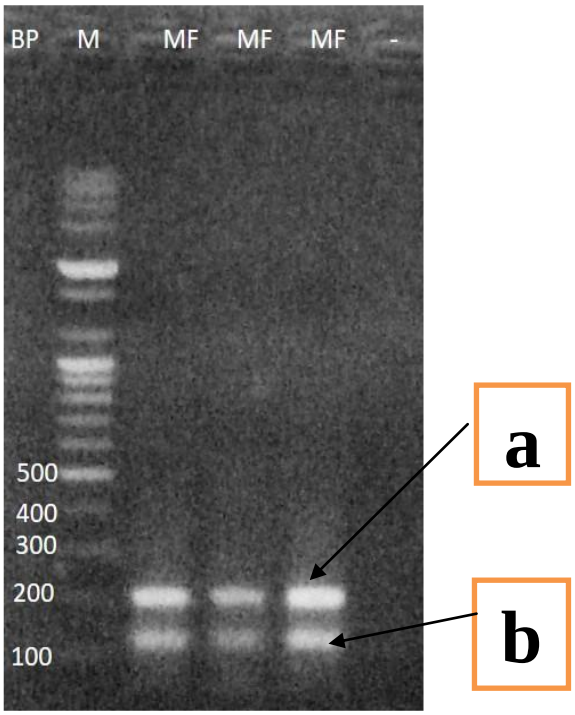

Figure 1.0 Gel image of *Mangifera indica* flower.

Many characteristics of sleep and sleep-wake regulation differ greatly amongst people, but are relatively consistent within particular groups. Despite the fact that sleep is a very diverse phenotype, several components of sleep have a high rate of variance even among healthy people (despite a very slim range of consideration). Figure 1.0 shows the gel image indicating the presence of prep1 gene in *Mangifera indica* (flower). The flower among other plant parts was the best option for this study because of the assertion that PreP1 isoforms are expressed in all tissues with highest expression levels in flowers and siliques [4], [9]. The addition of the isoforms gave a very clear band at exactly the region that corresponds to the molecular weight of two primers confirming the said assertion. Although this study could not analyze other plant parts for the possible presence of PREP1 gene. However, knowing that *Mangifera indica* flower contains a significant amount of PREP1 gene, this means that the said plant sample will be a good inhibitor to the overexpression of MEIS1 gene which happen to cause insomnia as stated in the introduction and this in turn will help defeat the numerous adverse effects associated with the use of synthetic sedatives.

4. CONCLUSION

The present study examined the presence of prep1 gene which is responsible for ameliorating the effects of insomnia, depression and other mental diseases in the flowers of *Mangifera indica*. Prep1 gene was found in significant amount in the flower of *Mangifera indica.* This is an indication that the said plant part will have a high tendency to inhibit the overexpression of the MEIS1 gene which is the major cause of insomnia complaints.

The high presence of PREP1 gene in *Mangifera indica* flower is a step in the right direction to finding a possible remedy for insomnia and this also emphasizes the need to for a more detailed study towards understanding PREP1 gene mechanism of action as this is crucial for further and future nutraceutical usage due to the vast spectrum of pharmacological effects and value to human health and their well-known antianxiety and sedative effects.

REFERENCES

[1] Al-Snafi, A. A review of chemical constituents and pharmacological activities of *Arachis hypogaea*. *International journal of pharmaceutical Research Scholars* (IJPRS) 2014(4) 1-1.

[2] Allen RP, Barker PB, Horska A, Earley CJ.. Thalamic glutamate/glutamine in restless legs syndrome: Increased and related to disturbed sleep. *Neurology,* 2014(80**)** 2028-2034.

[3] Baglioni C. Insomnia as a predictor of depression: a metanalysis of longitudinal epidemiological studies. *Journal of Affective Disorder,* 2011 (135)10-19.

[4] Bhushan S, Staahl A, Nilsson S, Lefebvre B, Seki M, Roth C et al. Catalysis, Subcellular localization, expression and evolution of the targeting peptides degrading protease, AtPrep2. *Plant Cell Physiology.* 2015 (46) 985-996.

[5] Brandon P, Steven G. Common sleep disorders: a wide array of conditions. 2019 http://www.verywell.health.com : overview of common sleep disorders.

[6] Cappuccino FP, Delia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: A systematic review and meta-analysis. *Diabetes* care 2010 (33) 414-420.

[7] Carol D. Understanding insomnia treatments. EebMD medical Reference: 2019 "Insomnia and sleep disorders".www.webmd.com/sleep- disorders/understanding- insomnia-treatments.com

[8] Goldman-Mellor S, Gregory AM, Caspi A, Harrington H, Parsons M, Poulton R. Mental health antecedents of early midlife insomnia: evidence from a four-decade longitudinal study. Sleep.: 2014 (37):1767–75.

[9] Hans GB, Elzbieta G. Functional and Structural Studies of the Presequence protease, PREP1. *Licentiate thesis*-department of Biochemistry, Stockholme University. 2014 Pg 1.

[10] Hergens TA, Kaleth AS, Edward ES, Butner KL. Association between sleep disorders, obesity and exercise: a review. *National science sleep* 2014 (5) 27-35.

[11] Kelly JM, Bianchi MT. Mammalian sleep genetics. *Neurogenetics* 2012(13) 287- 326.

[12] Laurent A, Calabrese M, Warnats H, Yaspo M. Chlpseq and RNA- sequence analysis identify components of the Wnt and Fgsignalling pathways as Prep1 target genes in mouse embryonic stem cells. 2014 PLoS ONE

[13] Lind MJ, Aggen SH, Kirkpatrick RM, Kendler KS, Amstader AB.A longitudinal twin study of insomnia symptoms in adults. *Sleep* 2015 (38) 1423-1430.

[14] Monier A. Traditional medicinal plants of Nigeria: an overview. *Agricultural and biology journal of North America.* ISSN Online: 2016. 2151-7525 I

[15] National Center on Sleep Disorders Research. National Institutes of Health Sleep Disorders Research Plan.Available 2011 :https://www.nhlbi.nih.gov/health/prof/sleep/201101011 NationalSleep

[16] Nilsson PM, Nilsson JA, Hedblad B, Berglund G. Sleep disturbance in association with elevated pulse rate for prediction of mortality- consequences of mental strain. *Journal of International Medicine* 2011 (250) 521-529.

[17] Schutte EC. Targeted resequencing and systematic in vivo functional testing identify rare variants in MEIS1 as significant contributors to restless legs syndrome. *American Journal of Human Genetics.* 2014 *(*95) 85-95.

[18] Spiengelhalder K. Magnetic resonance spectroscopy in patients with insomnia: a repeated measurement study. 2016 PLOS one 11, e0156771.

[19] Velumani A, Selvi V. Gas chromatography and mass spectroscopic analysis of bioactive constituents in *Mangifera indica* leaves extract. *jurnal of advanced scientific research* 2016 (4) 96-100.

[20] Wing YK.. Familial aggregation and heritability of insomnia in a community-based study. *Sleep Medicine.* 2012 (13**)**: 985-990.

[21] Winkelmann J. Genome-Wide association study identifies novel restless legs syndrome susceptibility loci on 2p 14 and 16q 12.1 2011 PLOS Genet.