

The Effect of Prolonged Consumption of Aqueous Extract of *Raphia Hookeri* Pulp Fruit on Selected Immune Parameters on Male Wistar Rats

Egbono, F. F¹, Mene A. E², Nwiko K. M³

Lecturer, Department of Human Physiology, Faculty of Basic Medical Sciences¹

Lecturer, Department of Medical Biochemistry, Faculty of Basic Medical Sciences²

Department of Human Physiology, Faculty of Basic Medical Sciences³

College of Health Sciences, University of Port Harcourt, Choba, Nigeria

frank.egbono@uniport.edu.ng¹, alexanda.mene@uniport.edu.ng², martins.nwiko@uniport.edu.ng³

Corresponding author: Egbono, F. F

Abstract: This research aims to ascertain the effect of oral administration of a aqueous fruit extract of *Raphia Hookeri* on selected immune parameters in male wistar rats. The fruits were obtained from Emoh community in Abua/Odual L.G.A, Rivers State, A total of 28 apparently healthy male rats weighing between 137-190g were divided into four groups, 7 rats in each group. Group 1 served as the control were given feed and water, group 2 given 500mg/kg, group 3 given 1000mg/kg, group 4 given 2000mg/kg of the extract for 28 days and blood samples collected. Laboratory test done and statistical analysis expressed as mean± SEM using Statistical Packages for Social Sciences (SPSS version 24). The result for IgA when compared the treated groups and control, there was slight variation in values but the result is not significant at $P < 0.05$, there were marginal variation in IgE, when compared the treated groups with the control, the result showed no significant difference, there was a significant decrease in group 2 for IgM, also a slight variation in group 3 and 4 when compared with the control not statistically significant at $P < 0.05$. Very plausible to state that there is no proportional effect of the aqueous extract on Immunoglobulin A (IgA), Immunoglobulin E (IgE). There was a significant decrease in Immunoglobulin in (IgM) level noticed in group 2 and no significant difference in groups 3 and 4. The findings of the study has proven that prolonged consumption of *Raphia Hookeri* fruit extract boost immunity via immunoglobulins in male wistar rats.

Keywords: Prolonged Consumption, Aqueous Extract, *Raphia Hookeri*, Immune parameters, Wistar Rats

I. INTRODUCTION

Immune parameters such as immunoglobulins play critical role in determination of status of immunity and its related medical conditions. Immunoglobulins also called antibodies, are proteins that the immune system makes to fight germs, such as viruses and bacteria. When the body is exposed to germs, the body makes unique antibodies that are specifically designed to destroy only those germs. Immunoglobulins blood test measures the amounts of IgM, IgG, IgD, IgA and IgE in the blood and help diagnose different types of health conditions that may affect the immune system but an immunoglobulins test usually measures three main types of immunoglobulin (Ig) antibodies that do different jobs to protect the body such as **IgM** antibodies are the first immunoglobulins the body makes after exposure to germs. They provide short-term protection while the body makes other antibodies. IgM antibodies are in the blood and lymph fluid. **IgG** antibodies are very important for fighting infections from bacteria and viruses. Most of the immunoglobulins in the blood are IgG. Some IgG antibodies are all in the body fluids. The body keeps a "blueprint" of all the IgG antibodies, that way, if the body is exposed to the same germs again, the immune system can quickly make more antibodies. **IgA** antibodies protect the respiratory tract and digestive system from infections. There are IgA antibodies in the blood, saliva, and gastric juices. Providing natural solutions to immunodeficiency and the likes over the years remains an issue and hence scientific investigation of plants on the benefits of improving the immunity is very imperative at this point in human history since the orthodox medicine seems expensive and most times ineffective.

Raphia Hookeri plant is one of such plants whose fruit mesocarp (pulp) commonly called Ogbusi when processed and consumed as staple food by the people of Abua/Odual LGA, Rivers state, Niger Delta Region, southern Nigeria is hypothesized to have ameliorative effect on hyperlipidaemia, boost immunity, inhibit plasma glucose, reduce blood pressure and boost haematopoiesis, etc (Egbono et al, 2023). The Raphia hookeri plant belongs to the family of Raphia palm trees and are found in abundance in the south-eastern southern part of Nigeria, especially in the southern part and considered edible in Emoh Community, Rivers state, Abua/Odual LGA and not edible in other parts which made its consumption rate low or none in such parts (Egbono et al, 2023). The boiled fruit pulp is commonly called 'Ogbusi' by the Abua people and mostly eaten with tapioca (processed cassava) commonly known as 'Ataka' by the Abua people of Rivers state in Nigeria (Egbono et al, 2023). Raphia Hookeri fruit pulp is a good source of phytochemicals and some micronutrients and is locally consumed as a snack (Tatianan et al, 2023). Its fruit is large, cone-shaped with a single hard nut having an outer layer of overlapping reddish brown scales and in-between the outer layer of scales and the hard seed is a yellow, mealy, oil-bearing mesocarp or pulp (Mbaka et al., 2012). Similarly, Ndon (2003) described raphia hookeri fruit as large, cone-shaped with a hard nut having an outer layer of rhomboid-triangular and overlapping reddish-brown scales. Between the outer layer and the seed, is a yellow, oil-bearing mesocarp or pulp (Ndon, 2003). The pulp extract of Raphia hookeri was shown to contain vitamins C and E, carotenes, niacin, alkaloid, saponins, flavonoids and phenols which explains its antioxidant activity (Edem et al., 1984; Akpan and Usoh, 2004; Dada et al., 2017). Flavonoids and tannins as phenolic compounds in plants are a major group of compounds that act as primary antioxidants by scavenging free radicals (Polterait, 1997).

The pulp has been reported to contain useful and therapeutic nutrients and chemicals. It is hard and often boiled before consumption. The oil processed from this plant is used for cooking and making margarine while the pulp is usually consumed with boiled cassava Mbaka, et al, 2012. Given its hard and relatively dry nature attributed to its high fiber content, it could be conveniently processed into flour, as an alternative form for consumption or added to pastries that are less diversified in nutrients. The pulp is known by locals as an appetizer and aphrodisiac (Mphoweh et al, 2009). Many uses it for medicinal purposes and it has been reported to contain phytochemicals with antimicrobial properties (Ogbuagu, 2008). The investigation carried out by Ogbuagu, 2008 showed that the pulp has higher concentrations of vitamin E ($1.04 \text{ mg} \cdot 100 \text{ g}^{-1}$), niacin ($0.2 \text{ mg} \cdot 100 \text{ g}^{-1}$), alkaloid ($5 \text{ g} \cdot \text{kg}^{-1}$), saponins ($3.6 \text{ g} \cdot \text{kg}^{-1}$), flavonoids ($4 \text{ g} \cdot \text{kg}^{-1}$) and phenols ($4.1 \text{ g} \cdot \text{kg}^{-1}$) than the seed, but the seed has higher values of vitamin A ($0.16 \text{ mg} \cdot 100 \text{ g}^{-1}$), thiamine ($0.07 \text{ mg} \cdot 100 \text{ g}^{-1}$), riboflavin ($0.07 \text{ mg} \cdot 100 \text{ g}^{-1}$), nitrates ($3.05 \text{ mg} \cdot 100 \text{ g}^{-1}$) and nitrites ($0.29 \text{ mg} \cdot 100 \text{ g}^{-1}$), and of the toxic elements: lead ($0.03 \text{ } \mu\text{g} \cdot \text{g}^{-1}$), mercury ($0.04 \text{ } \mu\text{g} \cdot \text{g}^{-1}$), arsenic ($0.23 \text{ } \mu\text{g} \cdot \text{g}^{-1}$) and cadmium ($0.04 \text{ } \mu\text{g} \cdot \text{g}^{-1}$) than the pulp and the pulp and seed of *R. hookeri* are non-toxic and can serve as food as well as in medicine.

Investigations carried out by Edem et al, 1984 to determine the chemical composition of the fruit of the raffia palm (*Raphia hookeri*: Family, Palmaceae or Palmae) and the peel and pulp (edible portion) were analysed. The effect of boiling on the chemical composition of the pulp was also investigated, it revealed that the peel contained more moisture (62.4%) than the pulp (38.0%) in terms of wet weight. Again, the protein and ether extract contents of the peel were found to be 3.2% and 1.8% of dry material, respectively. The ash content was 5.5%. Crude fibre gave a very high value of 70.3% for the peel, but the carbohydrate content was low (19.3%). There were decreases in the values of some nutrients after boiling the edible pulp of the fruit. Protein content decreased from 6.1% to 4.4% upon boiling. Ether extract and carbohydrate contents decreased from 11.8% to 11.3% and from 61.4% to 58.8%, respectively. Boiling increased the crude fibre and ash contents of the pulp from 17.7% and 3.0% to 21.2% and 4.3%, respectively. The calorific value decreased from 380.5 kcals to 354.7 kcals. Also revealed that tannin content was highest of all the toxic substances evaluated, there was a decrease from 597 to 360mg/100g on boiling. The peel contained 234mg/100g tannins and 24.3mg/100g hydrocyanic acid. Boiling the pulp resulted in reduction of the HCN from 12.4 to 9.2mg/100g, phytic acid from 1.0 to 0.4mg/100g, and oxalate from 26.4 to 17.6mg/100g. The peel had more oxalate (39.6mg/100g) and cyanide (24.3mg/100g) but less phytic acid (0.6mg/100g) than the pulp (Edem et al, 1984). Also, ascorbic acid and carotene contents decreased upon cooking the pulp from 63.0mg/100g and 33.4 $\mu\text{g}/100\text{g}$ to 28.3mg/100g and 10.6 $\mu\text{g}/100\text{g}$, respectively. The peel had an ascorbic acid content of 37.2mg/100g and carotene content of 8.6 $\mu\text{g}/100\text{g}$. Also Calcium, potassium, sodium and phosphorus decreased with cooking, while magnesium, zinc and iron contents were increased. Potassium had the highest level followed by calcium. The pulp had (mg/100 g): K, 1075; Ca, 875; Mg, 315;

Zn, 9.6; P, 76.8; and Na, 16. The peel had (mg/100 g): Ca, 250; Mg, 450; K, 700; Na, 8; Zn, 3.5; and P, 37.7. Copper, chromium and cobalt were not detected in the fruit (Edem et al, 1984).

The essence of this study is to provide data on the effect of prolonged consumption of *Raphia Hookeri* fruit on some Immunological parameters such as Immunoglobulins M (IgM), IgE and IgA on male wistar rats. Assessment and knowledge of immunoglobulin structure and classes is also important for selection and preparation of antibodies as tools for immunoassays and other detection applications. There are insufficient amounts of all classes of immunoglobulins, or they are absent. The presence of low IgA may be associated with recurrent diarrhea and lung and sinus infections. Low IgG is associated with pyogenic infections, and a high IgE may be found in parasitic infections. Justiz Vaillant & Ramphul. (2022). The purified immunoglobulin can treat many immunological problems, including antibody deficiencies, severe combined immunodeficiency disorders (SCID), multiple sclerosis, myasthenia gravis, Kawasaki disease, systemic lupus erythematosus (SLE), organ transplantations, and many others. Vince et al, 2018. Due to little or no scientific report/finding about the medicinal benefits of consuming fruits of this plant and the high rate of consumption of this fruit by people of Emoh community and Abua/Odual LGA of Rivers State, Niger Delta region, southern Nigeria prompted the lead researcher who is from Emoh community and others to carry out this research work to ascertain among other aspects the effect of this fruit on immune parameters after prolonged consumption of *raphia hookeri* fruit pulp extract for twenty eight days.

II. MATERIALS AND METHODS

2.1 Materials

1. Animal cage made of bowl and wire gauze, 2. Feeding and drinking plates, 3. Brooms and parker, 4. Disinfectant, 5. Dry saw dust, 6. Animal feed, 7. Laboratory coats, 8. Hand sanitizers, 9. Face mask, 10. Masking tape, 11. Weighing balance, 12. Baskets, 13. Hand gloves, 14. Water, 15. Hand towel

2.2 Study Design

The animal feed finisher mash and water were to the control (group 1) and the extract was administered to the other groups (2,3,4) for twenty-eight (28) days after they were all acclimatized for fourteen (14) days in the university of Port Harcourt animal house.

GROUP	DOSE OF EXTRACT	MODE OF ADMINISTRATION
Group 1 (control)	water and feed only	Orally
Group 2	Low Dose 500mg/kg of AFERH	Orally
Group 3	Moderate Dose 1000mg/kg of AFERH	Orally
Group 4	High Dose 2000mg/kg of AFERH	Orally

Sample Collection

Raphia hookeri fruit mesocarp (pulp) used for this study was purchased from the local market called Ayeeziin Abua/Odual LGA, Rivers State, Nigeria,

Extract Preparation of *Raphia Hookeri* (Rh) Fruit

Maceration

Maceration method was used for extraction. The fruits were air dried in order not to kill the active ingredients, then it was finely crushed and soaked in a maceration jar and allowed to stand for 72 hours with a continuous agitation to enable a good yield, after which it was filtered and then filtrate was mounted on a water bath to evaporate the liquid content at temperature of 65 degree Celsius. After evaporating, the weight of the extract was taken and it was stored for use.

2.3 Sampling Method

The rats were sacrificed under anesthesia with diethyl ether. Blood samples were also collected via venous puncture with syringes and needles and transferred accordingly in a well labeled Ethylenedimethyltetraacetic acid EDTA sample bottles for laboratory analysis.

2.4 Immunoglobulin Test

Test Analysis

IgA quantitative turbidimetric assay for the measurement of IgA human serum or plasma.

Anti-human IgA antibodies form insoluble complexes when mixed with samples containing IgA. The Scattering light of the immunocomplexes depends of the IgA concentration ion the patient sample. And can be quantified by comparison from a calibrator of known IgA concentration.

Reagents Compositions

IgA antibodies anti human IgA tris buffer 20 mmol, pH 8.2 sodium azide 0.95g/l, Plasma protein Muticalibrator, Protein Calibrator, Optional, Ref: 391005.

Precautions: The reagent contains sodium-azide 0.95 g/L.

Avoid any contact with skin or mucous.

Samples

Fresh serum and EDTA or heparinized plasma. IgA in Serum or plasma is stable 7 days at 2-8⁰C or 3 months at – 20⁰C. Samples with presence of fibrin should be centrifuged before testing. Highly hemolyzed or lipemic samples are not suitable for testing.

Material Required

Thermostatic bath at 37⁰C

Spectrophotometer or photometer thermostatable at 37⁰C

Capable to read at 340 + 20nm

Cuvettes with 1cm pathlength.

Pipettes to measurers reagents and samples

PROCEDURE

1. Prewarm the reagents and the photometer (cuvette holder) to 37^oc.
2. Using distilled water Zero the instrument at 340nm.
3. Pipette into cuvette
4. Mix well and insert the cuvette into the photometer. Record the absorbance (A) after 2 minutes of the samples or calibrator addition.

Sample / calibrator	7 μ l
Reagents (R1)	1.0ml

Calculation

Plot the different absorbance values (A) against the IgA concentration of each calibrator dilution. IgA concentration in the samples is calculated by interpolation it's (A) value in the calibration curve.

Principles

IgM quantitative turbidimetric assay^{1,2} for the measurement of IgM in human serum or plasma.

Anti-human IgM antibodies form insoluble complexes when mixed with samples containing IgM. The scattering light of the immunocomplexes depends of the IgM Concentration in the patient sample. And can be quantified by comparison from a calibrator of known IgM concentration

Reagents Composition

IgM antibodies-human IgM, tris buffer 20 mmol/L pH 8.2. Sodium Azide 0.95 g/l. Plasma protein muticalibrator. Protein calibrator optional .Ref:391005

Precautions: The reagents contains sodium azide 0.95 g/L. avoid any contact with Skin or mucous.

Samples

Fresh serum and EDTA or Heparinized plasma. Igm in serum or plasma is stable 7days at 2-8^oc or 3 months at -20^oC.

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Samples with presence of fibrin should be centrifuged before testing. Highly hemolyzed or lipemic samples are not suitable for testing

PROCEDURE

1. Prewarm the reagents and the photometer (Cuvette holder) to 37°C.
2. Using Distilled water Zero the Instrument at 540nm.
3. Pipette into cuvette
4. Mix well and insert the cuvette into the photometer. Record the absorbance (A) after 2 minutes of the sample or calibrator addition.

Samples / calibrator	7pl
Reagents (R1)	1.0ml

Calculation

Plot the different absorbance values (A) against the IgG concentration of each calibrator dilution. IgG concentration in the sample is calculated by interpolation of its (A) value in the calibrating curve.

Principle

IgG quantitative turbidimetric assay for the measurement of IgG in human serum or plasma. Anti-human IgG antibodies form insoluble complexes when mixed with sample containing IgG. The scattering light of the immunocomplexes depends on the IgG concentration in the patient sample, and can be quantified by comparison from a calibrator of known IgG concentration.

Reagents Composition

IgG antibodies anti-human IgG this, this buffer 20 mmol/L, pH 8.2 sodium azide 0.95 g/L. Plasma protein Calibrator. Optional, Ref 3910005.

Precaution: The reagent contains sodium azide 0.95g/L avoid any contact with skin mucous.

Samples

Fresh serum And EDTA or Heparinized plasma IgG in serum or plasma is stable 7 days at 2-8°C or 3 months at 20°C. samples with presence of fibrin should be centrifuged before testing. Highly hemolyzed or lipemic samples are not suitable for testing.

Material Required

Thermostatic bath at 37°C.

Spectrophotometer or photometer thermostatable at 37°C capable to read at 540±20nm

Curvettes with 1cm pathlength.

Pipettes to measure reagent and samples.

Procedure

1. Prewarm the reagent and the photometer (cuvette holder) to 37°C.
2. Using distilled water zero the instrument at 540 nm.
3. Pipette into a cuvette
4. Mix well and insert the cuvette into the photometer. Record the absorbance (A) after 2 minutes of the sample or calibrator addition.

Samples/calibrators	7µl
Reagent (R1)	1.0ml

Calculation

Plot the difference absorbance value (A) against the IgG concentration of each calibrator dilution. IgG concentration in the sample is calculated by interpolation of its (A) value in the calibration curve.

Statistical Analysis

Data analysis from the study will be expressed as mean± Standard Error Median (SEM). The analysis will be done using Statistical Packages for Social Sciences (SPSS version 24).

III. RESULTS

1: Effect of administration of aqueous Fruit extract of *Raphiahookerion* Immunoglobulins (IgA), (IgE)and (IgM) in male Wistar rats

Group and Treatment	IgA (mg/dL)	IgE (IU/L)	IgM(mg/dL)
Group 1: Control Group	39.50 ± 0.50	279.00 ± 9.00	36.00 ± 2.00
Group 2: Low Dose treated (500mg/kg b.w AFERH)	38.25 ± 0.63	278.25 ± 7.27	32.50 ± 0.96 ^a
Group 3: Medium Dose treated (1000mg/kg b.w AFERH)	38.66 ± 0.88	320.33 ± 33.38	34.66 ± 0.33
Group 4: High Dose treated (2000mg/kg b.w AFERH)	38.00 ± 1.29	273.50 ± 11.92	33.25 ± 0.85

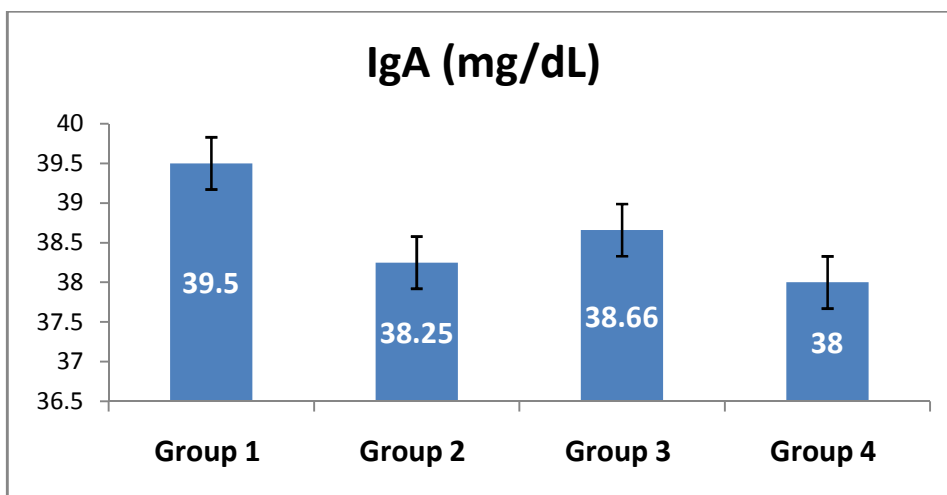


Figure 1: Effect of administration of aqueous fruit extract of *Raphiahookerion* IgA in male Wistar rats

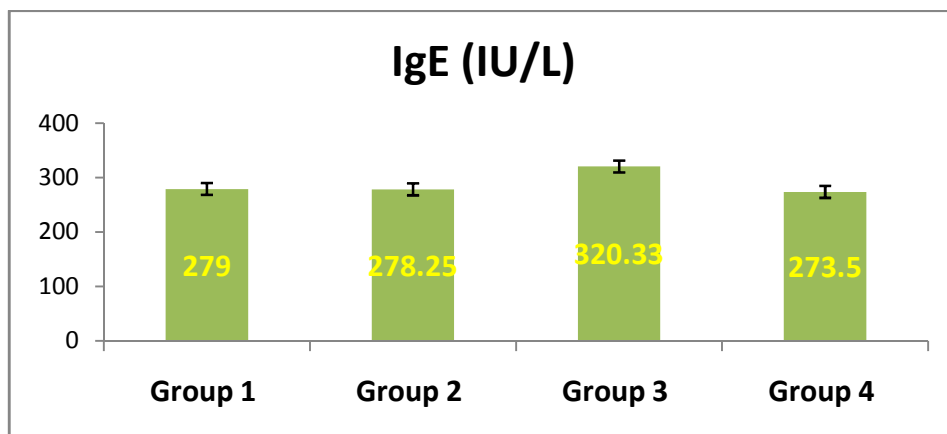


Figure 4.2: Effect of administration of aqueous Fruit extract of *Raphiahookerion* IgE in male Wistar rats

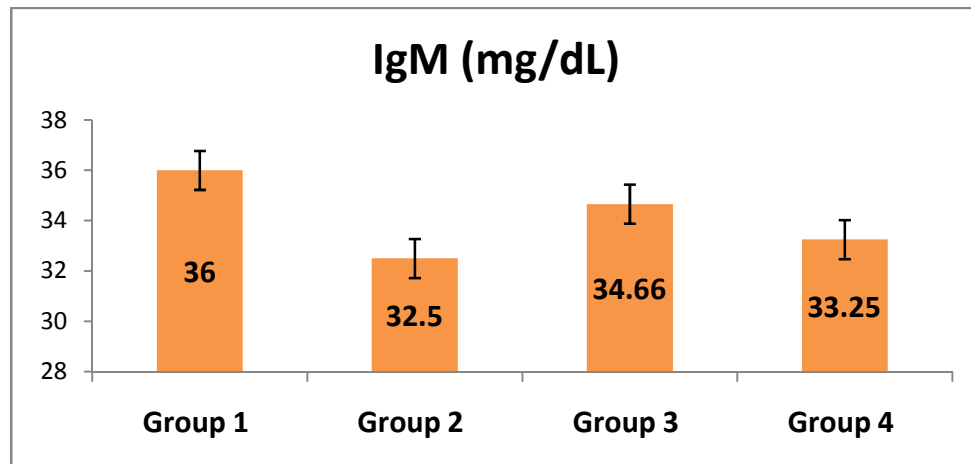


Figure 3: Effect of administration of aqueous fruit extract of *Raphiahookerion* IgM in male Wistar rats

IV. DISCUSSION

The result indicates non-significant ($p < 0.05$) changes in the mean level Immunoglobulin A (Ig A) across all the test groups when compared with that of the control group mean value. Although from the value in the result, there was marginal non-uniform decrease in the IgA levels of all treated groups of rats. This non uniform decrease could be due to the differences in dose concentration administered to the respective groups. The presence of low IgA may be associated with recurrent diarrhea and lung and sinus infections and a high IgE may be found in parasitic infections. Justiz& Ramphul. (2022).

The purified immunoglobulin can treat many immunological problems, including antibody deficiencies, severe combined immunodeficiency disorders (SCID), multiple sclerosis, myasthenia gravis, Kawasaki disease, systemic lupus erythematosus (SLE), organ transplantations, and many others (Vince et al, 2018). Furthermore, The Level of IgE as can be seen also showed non-significant ($P < 0.05$) variation in the groups treated with low dose, medium or moderate and high dose respectively when compared to the control groups mean value. Hyperimmunoglobulinemia E syndrome (HIES) (Rapini et al., (2007). The result shows that there were variation of IgM mean values in group two, treated with low dose of extract (500mg/kg.bw) that is, there is a significant decreases level when compared to that of the control group. This decrease in level could be due to fact that when all B cells are stimulated, they secrete first, IgM before others switch to produce IgG, IgA, or IgE. Meanwhile, the variation of the treated groups were not statistically significant. Selective IgM deficiency (SIgMD) has been reported in Western countries and is often associated with severe or recurrent infections, autoimmunity, allergies, and malignancies; SIgMD appears to be more common than originally realized (Louis and Gupta, 2014). The European Society for Immunodeficiencies (ESID) registry defines primary SIgMD as a serum IgM level repeatedly below 2 standard deviations(SDs) from the mean level for age with normal levels of the serum IgA, IgG, and IgG subclasses; the absence of T-cell defects; normal vaccination responses; and the absence of causative external factors (Janssen et al.,2019).When these criteria are completely fulfilled, this condition is referred to as “truly selective primary IgM deficiency” (true SIgMD). Most IgA patients remain asymptomatic their whole life. Coincidentally, they are often diagnosed during routine laboratory screening. However, some patients present with different complaints and clinical phenotypes, mainly with recurrent sinopulmonary infection, allergies, autoimmune diseases, gastrointestinal disorders, malignancies, and other severe complications (Yazdani et al, 2017). The most common manifestations associated with selective IgA deficiency are the recurrent pulmonary infections caused by extracellular encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. More severe symptoms occur when selective IgA deficiency is combined with IgG2 and IgG3 subclass deficiency (Hammarström et al., 2000).

Autoimmune diseases have been observed in roughly 20 to 30% of patients with selective IgA deficiency. With selective IgA deficiency, allergic conjunctivitis, eczema, rhinitis, urticaria, food allergy, and asthma are commonly noted. There is an association of IgA deficiency with type 1 diabetes (Giza et al, 2016). Type 1 diabetes, SLE, celiac

disease, and Graves disease can share the same 8.1 haplotype as IgA deficiency. Scores of other autoimmune maladies also share an association (Singh et al., 2014, Gulez et al., 2009) It is thought that the overlap of genetic loci contributes to this association. Celiac disease shares a specific significance with IgA deficiency in that the paucity of IgA antibodies can lead to the misdiagnosis of celiac disease (See Evaluation - Special Note). The prevalence of IgA deficiency in celiac disease is about 2 to 2.5% (Méndez et al., 2021). Generally speaking, autoimmune disease is associated with IgA deficiency through various HLA markers such as A1, B8, DR1, DR3, DR7, DQ2 (8.1 haplotype), and DQ5. Celiac disease is associated with IgG deficiency, the 8.1 haplotype, and the HLA-DQBO2 allele. Incidentally, patients with concurrent celiac disease and IgA deficiency have a greater incidence of other autoimmune illnesses when compared to individuals with celiac disease alone. Some autoimmune diseases can have manifestations that can cloud or cover IgA deficiency necessitating an additional workup. Certain conditions like ataxia-telangiectasia can be problematic based on their multiple immunoglobulin deficiencies (IgA, IgG, and IgE are all decreased, unlike in IgA deficiency, where the IgG is normal). Patients with Hyper-IgM syndrome will present with decreased IgA as part of a hypergammaglobulinemia where only the IgM is elevated. These patients present with severe infections and hyperviscosity (due to the increased IgM) and are inherited in either an X-linked or autosomal recessive format (Qamar and Fuleihan, 2014). Selective IgA deficiency should be a possibility in every workup of an autoimmune disease. Hence, the finding of this study has proven that prolonged consumption of raphia Hookeri fruit extract boost the immune system in male wistar rats.

V. CONCLUSION

It is very plausible to state that there is no proportional effect of the aqueous extract on Immunoglobulin A (IgA), Immunoglobulin E (IgE) but there was a significant decrease in Immunoglobulin M (IgM) level notice when treated with a low dose (500mg/b.w) and no significant difference in medium and high dose which has ameliorate effective on hypergammaglobulinemia where a patient or individual with severe infections and hyperviscosity are inherited in either an X-linked or autosomal recessive format. The findings of the study has proven that prolonged consumption of Raphia Hookerifruit extract boost immunity via immunoglobulins in male wistar rats.

VI. RECOMMENDATIONS

It is recommended that;

More research should be made on Raphia Hookeri fruit using a higher dose than what was used in this research.

Due to limited resources available for this research, not every parameters was adequately considered as such, this work should be used as a stepping stone for further researches.

Raphia Hookeri fruit should also be experimented to ascertain the chemical components that had an effect on Immunoglobulin.

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