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Toxicological studies of the effects of ethanolic extracts of avocado (*Persea Americana Mill.*) seed flour on experimental animals

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Abstract

Ethanolic extract of Avocado (*Persea americana*) seed flour was assessed for histopathologic and haematological parameters of Albino rats treated with varying dosage (250, 500 and 1000mg/kg) of the extracts. The haematological parameters were RBC, PCV, Hb, WBC, PLT, MCV, MCH and MCHC with range of values 7.01 to 7.32x10^{6}mm^3, 43.40 to 46.80%, 14.86 to 16.12g/dl, 8.94 to 9.26x10^{3}mm^3, 89.40 to 94.60x10^3/mm^3, 61.93 to 63.97f1, 21.06 to 22.02pg and 33.17 to 34.73g/dl respectively. The seed extracts did not have adverse effects on the hematological parameters. Conclusively, these seeds should be harnessed and utilized for food and feed production.

Keywords: Ethanolic extracts; Avocado seed; Haematological; Albino rats; Histopathologic

1. Introduction

Persea americana (commonly known as avocado, avocado pear, or alligator pear) is native to Mexico and Central America, and a member of the flowering plant family Lauraceae (Bergh and Ellstrand 1986; Segovia *et al.,* 2018). Botanically, avocado fruit is a berry with a single large seed (Cowan and Wolstenholme, 2016). Mexico is the leading producer of avocados worldwide (Segovia *et al.,* 2018). Avocado has recently gained dramatic popularity (Rahmani *et al.,* 2017) and is often referred to as a "superfood" because of its unique nutritional and phytochemical composition compared to other fruits. This has led to an exponential increase in avocado consumption from 2.23 pounds per capita in 2000 to 7.1 pounds per capita in 2016 in the United States (Agricultural Marketing Resource Center, 2018). Considering their immense popularity and diverse biochemical content, avocados have also been extensively used in the food, nutraceutical, pharmaceutical, and cosmetic industries. In addition, their health-benefiting properties have been investigated in a number of preclinical and clinical studies in the last few decades.

It is a source of carbohydrate, protein, fiber, essential micronutrients for human consumption such as, polyphenols, fats, oils, vitamins (Vit. C, E, K, B1, B2, B6, B9) and minerals (P, Na, Mg, K, Fe and Zn) (Orhevba and Jinadu 2011; Oluwole *et al.,* 2013; Maitera *et al.,* 2014; Harborne & Williams, 2000; Pennington & Fisher 2009). Its low sugar content makes avocado very recommendable source of high-energy food for those who are diabetic. It is highly consumed in the world due to the presence of unsaturated lipids and its relevance in improving and maintaining healthy heart and circulatory system (Maitera *et al.,*2014).

There is a global tendency towards industrial fruit processing and, following such processes by-products are normally discarded. Seeds (endocarp) and peels (exocarp) being the by-products of the avocado industry are generally disposed of as wastes (Athaydes *et al.,* 2019). Thus, studies to investigate the benefits of these byproducts as sources of food supplements or medicinal products are needed (Ramos *et al.,*2004). Different parts of avocado pear are used in traditional medications for various purposes including as an antimicrobial. Exploring the possible dietary and therapeutic potentials of especially underutilized agro-food wastes will in addition reduce the possible environmental

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waste burden (Egbuonu *et al.,*2018). The seed of avocado is one of the under-utilized non-edible parts of the fruit, which are usually discarded as residues and can cause ecological problems. Exploring the possible dietary and therapeutic potentials of especially such underutilized wastes will in addition reduce the possible environmental waste burden (Shruti and Padma, 2015). Conducting a research on non-edible parts of fruits is an emerging trend, which may prove to be very profitable in the near future. Mostly, because it involves an important reduction in the production of by-products and the fact that the non-edible parts of many fruits like avocado have high levels of valuable bioactive compounds, particularly natural antioxidants (Vinha *et al.,* 2013; Mensah *et al.,*2015). Biological activities of the avocado seed such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, and recently amoebicidal and giardicidal activities have been reported (Noorul *et al.,*2016). Adeyemi *et al.*(2002) states that uses of avocado pear seed include use in the management of hypertension, diabetes, cancer and inflammation(Anaka *et al.,*2009). Several beneficial medicinal properties of compounds present in the avocado seed have been reported, which are related to the elevated levels of phenolic compounds (64 % in seed, 23 % in peel, and 13 % in pulp). In addition, the seeds and peels of avocado also contribute 57 % and 38 % of the antioxidant capacities of the entire fruit, respectively (Wang *et al.,*2010).

2. Material and method

2.1. Collection of sample and preparation

The avocado was obtained from the local market. The fruits were thoroughly screened to remove bad ones. The pulp and seed were manually separated and subjected to conditioning. The seeds were sliced and oven dried for 6 hours at 50 °C. After oven drying, the seeds were then milled to fine powder using Thomas-Wiley milling machine. The milled samples were stored in an air tight bottle till when needed for analysis.

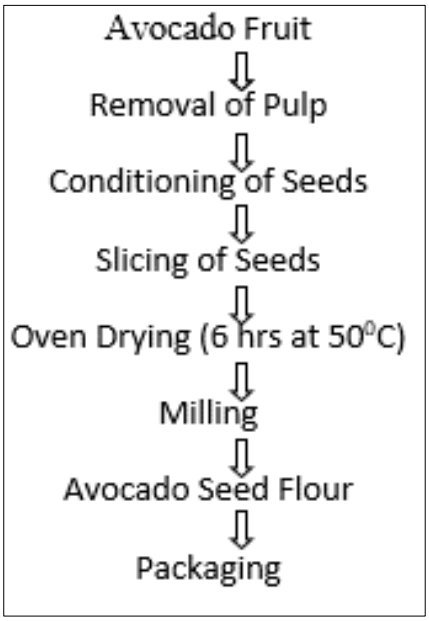

Figure 1 Flow chart for the processing of Avocado seed into Flour

2.2. Toxicological Studies

Acute Toxicity (LD_{50}) Evaluation of the extracts of the Avocado seed flour.

2.3. Preparation of extract

Preparation of the seed extract was done at the physiology laboratory, Department of Zoology and environmental biology, College of Natural Sciences Micheal Okpara University of Agriculture Umudike while the administration of the extract to the animals was done at the laboratory animal house of the department. Fifty (50) grams of the flour was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 60 ˚C for 48 hours. At the end of this period, the ethanol was evaporated at low temperatures first in a rotary evaporator and then taken to dryness in a hot air oven to obtain a crude extract for the sample. The extract was preserved in the refrigerator until needed.

2.4. Evaluation of the extracts for acute toxicity (LD_{50})

To evaluate the acute toxicity of the extracts, the new Lorke's method was adopted as was used by Orieke *et al.*(2019). For the extract, two test phases was carried out on a total of 21rats of both sexes purchased at the animal house of the Department of Zoology and environmental biology, College of Natural Sciences Micheal Okpara University of Agriculture Umudike. In the first phase, 9 rats divided into three groups (1, 2 and 3) was administered 10, 100, and 1000 mg/kg of the extract respectively. Following zero mortality recorded in the first phase, the study proceeded to the second phase where 9 rats also divided into three groups of 3 rats each and served as group 4,5 and 6. Group 4 and 5 was administered 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively while the highest dose administered (5000 mg/kg) was repeated on another group of 3 rats. All treatments was single dose and via oral route.

Mortalities recorded was noted within 24 hours of treatment and a further 7days for possible delayed toxicity. Acute toxicity values of the samples was calculated using Lorke's formula expressed as:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: LD_{50} = Lethal Median Dose
LD_0 = Highest dose that gave no mortality
LD_{100} = Lowest dose that produced 100 % mortality.

2.5. Haematological Studies

2.5.1. Determination of Haematological Parameters

Haematological parameters including RBC, PCV, Hb, WBC, PLT, MCV, MCH and MCHC were obtained at once for each blood sample in an automated haematology analyzer (BC-2300 Mindray, China) following standard protocols outlined by the producer.

2.6. Histopathology

2.6.1. Tissue preparation

Sections of the liver and kidney were collected for histopathological examination. The samples were fixed in 10 % phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70 %, 80 %, 90 % and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bathe and incubated at 60 ˚C for 30 minutes. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

2.6.2. Slide Examination

The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were randomely taken using a Motic™ 5.0 megapixels microscope camera at x160 magnifications.

2.7. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 21 was used for analyzing data obtained from the experiment and all analyses were carried out in duplicates. Statistical significance ($p < 0.05$) was established using one-way analysis of Variance (ANOVA) models and means separated using Duncan Multiple Range Test (DMRT).

3. Results and discussion

3.1. Result of Acute Toxicity Evaluation

No mortality was observed in any of the test groups in the two phases of the test after 24h period of the acute toxicity study even at a maximum dose administration of 5000 mg/kg body weight. Further observation of the experimental animals for a 7-day period still did not produce any mortality. The test animals rather exhibited tendencies of sound

physical health and emotional stability. There were no significant changes in the behavior in relation to posture, mood and motor activity or convulsions throughout the acute toxicity evaluation period of the extract.

Table 1a Acute toxicity evaluation outcome in phase I

Groups	Dose (mg/kg)	Mortality	% Mortality	Observations
1	10	0/3	0.00	Zero mortality. Animals appear physically stable.
2	100	0/3	0.00	
3	1000	0/3	0.00	

Table 1b Acute toxicity evaluation outcome in phase II

Groups	Dose (mg/kg)	Mortality	% Mortality	Observations
1	1600	0/3	0.00	Zero mortality. Animals appear physically stable.
2	2900	0/3	0.00	
3	5000	0/3	0.00	

5000 mg/kg was repeated on another set of 3 animals and still no mortality was observed after 24 hours and a further 7 days.

LD_{50}> 5000 mg/kg

Table 2 Effect of Extract on Haematological Parameters

Treatment Groups	RBC (x106/mm3)	PCV (%)	Hb (g/dl)	WBC (x103/mm3)	PLT (x103/mm3)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	7.01±0.28^{a}	43.40± 2.07^{a}	15.04± 0.85^{a,b}	9.26±0.55^{a}	94.60±4.04^{a}	61.93± 0.63^{a}	21.46± 0.46^{a,b}	34.65± 0.81^{b}
Extract (250 mg/kg)	7.06±0.12^{a,b}	44.80± 0.84^{a,b}	14.86± 0.30^{a}	8.94±0.19^{a}	92.20±6.50^{a}	63.47± 0.53^{b}	21.78± 0.39^{a}	33.17± 0.45^{a}
Extract (500 mg/kg)	7.32±0.08^{b}	46.80± 0.87^{c}	15.94± 0.83^{b,c}	9.23±0.56^{a}	89.40±5.77^{a}	63.97± 0.82^{b}	21.06± 0.95^{a,b}	34.05± 1.31^{a,b}
Extract (1000 mg/kg)	7.32±0.20^{b}	46.4o± 1.34^{b,c}	16.12± 0.81^{c}	8.96±0.29^{a}	94.20±6.61^{a}	63.41± 0.76^{b}	22.02± 9.53^{b}	34.73± 1.01^{b}

Values are presented as mean ± standard deviation (n = 5), and values with different superscripts are significantly different (P<0.05) from any paired mean within the column.

As shown in table 2, assessment of the effect of avocado seed extract on hematological parameters of rats showed that avocado seed extracts caused a significant increase in the red blood cells (RBC) of the experimental rats. The results showed that the control rat had RBC value of 7.01 X 10^6 while the rats induced with an extract of 250 mg/kg had RBC value of 7.06 X 10^6, treatment groups with extract of 500 mg/kg had RBC value of 7.32 X 10^6, and the rat induced extracts of 1000 mg/kg had RBC value of 7.32 X 10^6. This result is an indication that avocado pear seed extract could increase the red blood cell count in albino rat. Packed Cell Volume (PCV) followed similar trend of increased value with increase in the quantity of ethanolic extracts of avocado seed. The PCV values obtained in this study were 43.40 %, 44.80 %, 46.80 % and 46.40 % for control rat, 250 mg/kg extract, 500 mg/kg and 1000 mg/kg extract treated rats respectively.

The control experimental rats had haemoglobin (Hb) value of 15.04 g/dl. It was also observed that 250 mg/kg ethanol extract of avocado seed treated rats had a decreased Hb value of 14.86 g/dl. Experimental rats treated with 500 mg/kg and 1000 mg/kg ethanol extracts of avocado seed had significant increase in their Hb value. Treatment groups with

1000 mg/kg ethanol extracts of avocado seed had the higher Hb value of 16.12 g/dl. The haematology results obtained in this study for avocado seed extracts could be compared with the effect (increase), but not with the values reported by Braii *et al* (2020), owing perhaps to the different methods and concentration of the extracts used in the individual study.

The White Blood Cell (WBC) count obtained in this study falls within the range of $8.94x10^3$ to $9.26x10^3$, however these values were higher than the range of values presented by Olufunke (2014) for WBC count of Wistar rats treated with ethanol extract of *Moringa oleifera* roots. Treatment of the experimental rats with ethanol extracts of avocado seeds caused significant reduction in the white blood cell counts of the rats. This trend is not expected as avocado seed contains some bioactive compound that showed antimicrobial, antiviral, and antitumor activities (Ejiofor *et al*., 2018).

Platelet (PLT) count decreased with increased in the concentration of ethanol extracts of avocado seed. The control treatment group had the highest platelet count of $94.60x10^3$ and the treatment group treated with 500 mg/kg of ethanol extract of avocado seed had the least value of $89.40x10^3$. These values fall below the platelet count of 112.00±8.32 present by Olufunke (2014) for Wistar rats treated with ethanol extract of *Moringa oleifera* roots.

There was significant ($p \leq 0.05$) increase in the Mean Cell Volume (MCV) of the experimental rats. The control group had MCV value of 61.93±0.63 fI, treatment group of 250 mg/kg extract had MCV value of 63.47±0.53 fI, group treated with 500 mg/kg had MCV value of 63.97.82 fI and group treated with 1000 mg/kg had MCV value of 63.41±0.76 fI. Olufunke (2014) recorded similar range of values (58.6 – 60.6 fI) for ethanol extract of *Moringa oleifera* treated wister rats. George *et al* (2020) also recorded similar value in the MCV of broilers fed with 1.5 % avocado seed meal.

There are significant difference ($p \leq 0.05$) in the mean corpuscular haemoglobin (MCH) of albino rats treated with ethanol extract of avocado seed. Treatment of rats with ethanol extract of avocado seed caused an increase in their mean corpuscular haemoglobin. This observation agreed with the finding of George *et al* (2020) who found range of values (22.40 to 22.67 pg) for broilers fed with 1 and 1.5 % avocado seed meal. Olufunke (2014) confirmed this trend by obtaining an MCH value of 19.7 pg for Wister rats treated with ethanol extracts of *Moringa oleifera* roots.

Introduction of ethanol extracts of avocado seed caused significant reduction in the Mean Corpuscular Hemoglobin Concentration (MCHC). The control group treated group had MCHC value of 34.65 g/dl, 250 mg/kg extract treated group had MCHC value of 33.17 g/dl, 500 mg/kg extract treated group had MCHC value of 34.05 g/dl and 1000 mg/kg extract treated group had MCHC value of 34.73 g/dl. It was observed that the MCHC value also increased with increase in the concentration of ethanol extract of avocado seed.

3.2. Histopathological Photomicrographs

3.2.1. Group 1 (Control) – Liver

Sections of the **liver** presented in this group showed the normal hepatic histomorphology for laboratory rodents. The tissues sections showed numerous normal hepatic lobules, containing normal hepatocytes arranged in radiating interconnecting cords around the central veins (V). The hepatic cords are separated by normal sized sinusoidal spaces. Normal structures of the portal triads (P) [hepatic vein; hepatic artery and bile ducts] were also observed. H&E x160

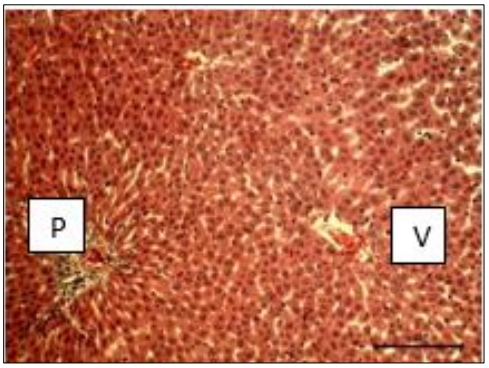

Figure 2 Liver tissue for the control group

Kidney

Sections of the kidney presented in this group showed the normal renal histo-architecture. Normal Glomeruli (G) in sea of normal renal tubules (arrow) suspended in a highly vascularized connective tissue meshwork were observed. H&Ex160.

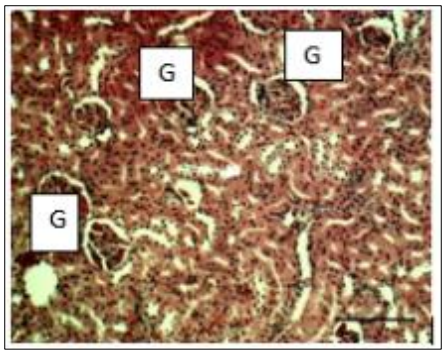

Figure 3 Kidney tissue for the control group

3.2.2. Group 2 – liver

Sections of the **liver** presented in this group showed the normal hepatic histomorphology for laboratory rodents. The tissues sections showed numerous normal hepatic lobules, containing normal hepatocytes arranged in radiating interconnecting cords around the central veins (V). The hepatic cords are separated by normal sized sinusoidal spaces. Normal structures of the portal triads (P) [hepatic vein; hepatic artery and bile ducts] were also observed. H&E x160

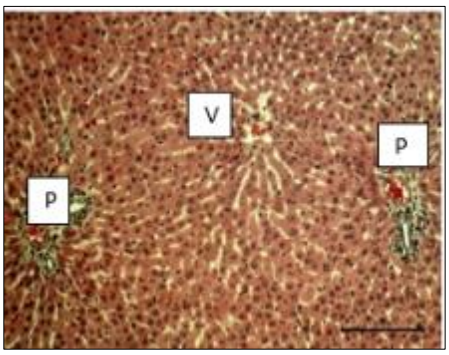

Figure 4 Liver tissue for the group treated with 250 mg/kg ethanolic extract

Kidney

Sections of the kidney showed the normal renal histo-architecture. See group 1 for details. Glomeruli (G); Renal tubules (arrow). H&Ex160.

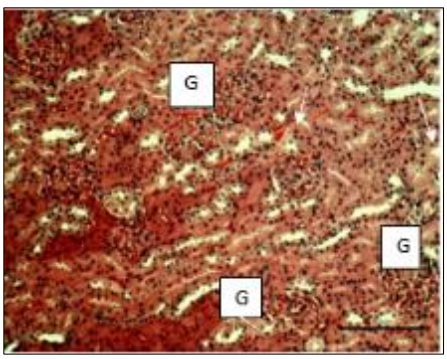

Figure 5 Kidney tissue for the group treated with 250 mg/kg ethanolic extract

3.2.3. Group 3 – liver

Sections of the liver presented on this slide showed the normal hepatic histo-architecture. See group 1 above, for detailed description. Central vein (V); Portal area (P). H&Ex160.

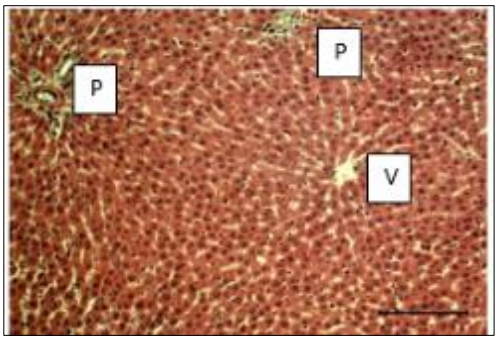

Figure 6 Liver tissue for the group treated with 500 mg/kg ethanolic extract

Kidney

Sections of the kidney showed the normal renal histo-architecture. See group 1 for details. Glomeruli (G); Renal tubules (arrow). H&Ex160.

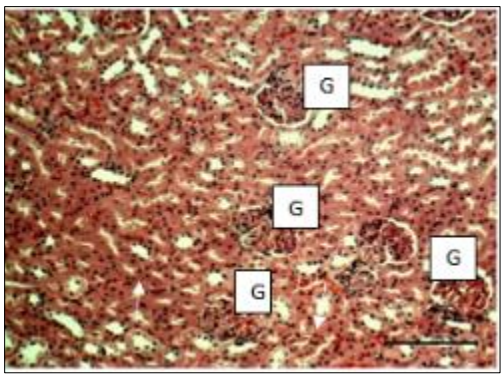

Figure 7 Kidney tissue for the group treated with 500 mg/kg ethanolic extract

3.2.4. Group 4 – Liver

Sections of the liver presented in this group showed mild periportal infiltration of inflammatory leucocytes (arrow). However, the hepatocytes were normal. Central vein (V); Portal area (P). H&Ex160.

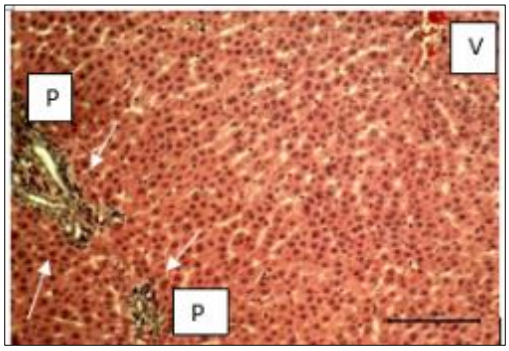

Figure 8 Liver tissue for the group treated with 1000 mg/kg ethanolic extract

Kidney

Sections of the kidney showed the normal renal histo-architecture. See group 1 for details. Glomeruli (G); Renal tubules (arrow). H&Ex160.

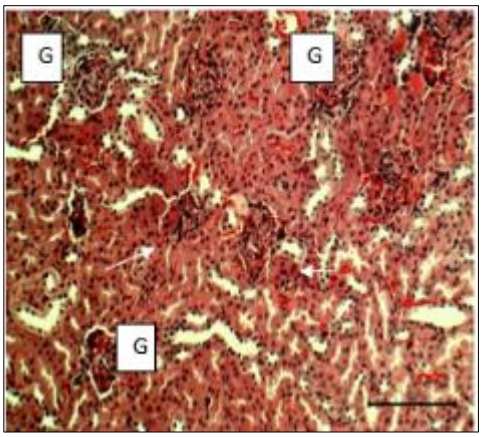

Figure 9 Kidney tissue for the group treated with 1000 mg/kg ethanolic extract

4. Conclusion

This work presented reliable and detailed information in terms of values obtained. The seeds were found to be edible and can be used in formulating diets having been subjected to various analysis.

The ethanol extracts of the avocado seed showed a positive effect on the levels of red blood cells, packed cell volume, mean cell volume and mean corpuscular hemoglobin but caused a reduction effect on Hemoglobin, white blood cells and mean corpuscular hemoglobin concentration.

Having analyzed the toxicological effects of the ethanolic extracts of the avocado seed flour it is recommended that there should be an inclusion of the seed flour in formulation of novel food products and feed for the health benefit of Man and Animals.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

This research work contains studies performed on experimental animals bred for the specific purpose of experimentation at the Animal house of the Department of Zoology and Environmental biology, College of Natural Sciences Micheal Okpara University of Agriculture Umudike.

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