EFFECT OF PERICARP OIL AND SEED OIL OF *PERSEA AMERICANA*ON THE PANCREAS OF ALLOXAN INDUCED DIABETIC MALE WISTAR RATS

ABSTRACT

There has been a continuous global rise in the epidemic of diabetes mellitus even with the massive investment in research on the subject**.** It poses a serious challenge to primary health care in developing countries, with negative consequences on the economy. This research is aimed at evaluating the effect of aqueous seed extract of *Persea ~~Americana~~ americana*on the pancreas of alloxan induced diabetic~~es~~ rats. Fifty six adult male Wistar rats were used for this study, 7 rats in each group.**Group A served as** Control and received food and water only. Experimental groups B to H were made diabetic by alloxan intraperitoneal induction at 200mg/kg body weight. **Group B received no further treatment. Group C was treated with** pericarp extract of fruit at a body weight dose of 100mg,, **Group D received an extract of fruit dose** at 200 mg/kg body weight; **Group E received extract of** dose at 200 mg/kg of body weight; **Group F received** fruit extract at the dose of 200 mg/kg body weight and fruit extract at the dose of 200 mg/kg body weight. **Group G received** Pear oil + Seed oil + Control drugs metformin while **Group H received** metformin (standard drug).The blood sugar and body weight of the rats was recorded at two weeks and four weeks. Our result~~s~~ show~~s~~ that significant decrease ($P<0.05$) in blood glucose were observed in all groups compared to Group B. Significant body weight increase was recorded in groups C, D and E compared to group B at $P < 0.05$. The findings indicates anti-diabetic effects of the extract which may be due to certain mineral elements and phytochemicals, and the increase in weight may be due to proper nutrient utilization probably induced by the avocado seeds and pericarp fruit extract. Avocado seeds may be of great benefit in the management of diabetis mellitus.

Key Words: Avocado pear, *Persea americana*, Alloxan, Diabetes, Serum glucose

INTRODUCTION

Diabetes mellitus (DM) is incredibly the world's quickest growing metabolic disorder and as the heterogeneity knowledge of this disorder becomes obvious so does the need for more appropriate and good therapies (Abdel MA, El-Feki M, 2020). DM is a condition that is pathological and results in chronic metabolic imbalances and non-physiologic changes in organic tissues (J. V. Hunt, M. A. Bottoms,2020). Oxidative stress play important roles in the aetiology of several terminal diseases including DM. Diabetes is linked wit building up of reactive oxygen species (ROS) which can cause oxidative damage, in heart, kidney, eyes, liver, small and large blood vessels and gastrointestinal system (Evans and Gold fire, 2020).

Increased level of glucose concentration directly increases hydrogen peroxide producedn by murine mesangial cells and lipid peroxidation of glomeruli and glomerular mesangial cells (~~,~~–T. Tuvemo, U. Ewald,2020). Hyperglycaemia supports glycosylation of circulating cells and

Formatted: No widow/orphan control

Formatted: Numbering: Continuous

Comment [Ma1]: Scientific specific name should start with lower case letter

Comment [Ma2]: Your style of reference must be consistent. Sometime, you used APA format and sometimes another format. Please be consistent. I am sure the call for submission indicates the reference format desired by the publisher. Kindly adopt the accepted format e.g. J.V. Hunt, 2020 is different from JV, Hunt, 2020

cellular protein and may introduce a series of autooxidative reactions in that culminate in accumulation of advanced glycosylation as end-products (AGE) in tissue protein. The AGE has an oxidizing potency and can support tissue destruction by free radicals (J. E. Hall, and A.C Guyton,2020). Furthermore, increased l peroxidation of lipids retards a membrane's function by reducing membrane fluidity nature and changing the activity of bound-membrane enzymes and receptors alike. Its end-products (lipid radicals and peroxides), are harmful to the cells in the body and connected with atherosclerosis and brain destruction, kidney, liver and other tissues alike.

Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus. The mechanism of alloxan action has been studied and can be properly characterized.,(T. Tuvemo, U. Ewald,2020). Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment. This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet cells, response to glucose even when high concentrations of glucose were used (,,–T. Tuvemo, U. Ewald, 2020). Furthermore, the alloxan action in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups (Evans and Gooldfire, 2020).

Comment [Ma3]: Full stop and coma should be removed from here

The International Diabetes Federation (IDF) reports that the prevalence of diabetes mellitus has reached epidemic levels globally. Recent estimates (Evans and Gooldfire, 2020) indicate that there were 366 million diabetics worldwide in 2020, and this number is expected to increase to 552 million by 2030. Impaired glucose tolerance in sub-Saharan Africa is expected to rise by 75.8%, from 26.9 million in 2010 to 47.3 million in 2030, which is more than double the predicted global increase of 37%. Mortality that was attributable to diabetes in sub-Saharan Africa was estimated in 2010 to be 6% of the total mortality, and this value had increased from 2.2–2.5% in 2000. The absolute and relative mortality rates are highest in the 20–39 year age-group, i.e., the most economically productive population.In Nigeria, which has over 250 tribes and different culture and food values, the prevalence values of diabetes have not been uniform, (Evans and Gooldfire, 2020) although the values range from 1–7% of the Nigerian population. Over 30 years, the prevalence of diabetes has steadily increased. Iloh et al. reported a prevalence of 3.9% for Imo state. However, a higher prevalence rates was reported in Port Harcourt (6.8%) by (Nyenwe et al. 2021). According to the estimates in 2020, by the Diabetes Association of Nigeria (DAN) estimates the diabetic population in Nigeria to be approximately 10 million, and approximately half of that number resides in the Lagos State because of its cosmopolitan nature These findings indicate that diabetes has become a major public health issue (Evans and Gooldfire, 2020).

Comment [Ma4]: This start another paragraph

Plants and plant products have been utilized in folkloric medicine in the treatment and management of disease conditions. Plants may act on blood glucose through different mechanisms. Some plants may contain insulin-like substances, inhibit insulinase activity or increase beta β-cells in the pancreas by activating the regeneration of these cells or some may serve as antioxidants by reducing the oxidative stress due to free radicals in the pancreas. *Persea americana* (avocado) is a tree that belongs to the laurel family, *Lauraceae*, and is one of the 150 varieties of avocado pear. This plant is indigenous to Central and South America, but it is now cultivated in the United States of America, Asia, parts of Europe, and Tropical Africa and is commonly known as the avocado pear, (J.L.Evans, I.D, Gooldfire, vol 23, 2020).The medicinal relevance of the various parts of this tropical plant is enormous. The effects of aqueous seed extracts of *Persea americana* on the blood pressure, plasma, and tissue lipids of albino rats were investigated by Imafidon and Amaechina, and their results suggested that the use of the aqueous seed extract of this plant in the treatment of hypertension might produce a favourable lipid profile. Alhassan and colleagues also evaluated the hypoglycaemic activity of *P. americana* aqueous seed extracts on alloxan-induced diabetic rats and concluded that the anti-diabetic effects of the extract might be due to certain mineral elements and phytochemicals. However, the work by (Okonta et al. 2020) suggests that *P. americana* can lower blood glucose levels in cases of mild hyperglycemia but not severe hyperglycemia. Edem et al. studied the effects of aqueous alligator pear seed extracts on normal and alloxan-induced diabetic rats, and their results suggested a restorative (protective) (J.L.Evans, I.D, Gooldfire, 2020)effect of the extract on pancreatic islet cells. The work of (Mahadeva et al., 2020) concentrated on the mechanism of the antidiabetic activity of *P. americana*. The insulin-stimulative and antioxidative effects of *Persea americana* were evaluated in streptozotocin (STZ)-treated rats. They found that the activities of pathophysiological enzymes such as serum aspartate transaminase (AST), serum alanine transaminase (ALT), and serum alkaline phosphatase (ALP) were altered in the serum of rats that had been treated with glyclazide, which was used as the standard reference drug, but not control rats. These results revealed the tissue protective nature of Persea americana fruits.

The pancreas is a long, soft organ in the upper left abdominal region. It sits below the liver, behind the stomach, and extends from the upper part of the small intestine to the spleen.

The main function of the pancreas is to produce chemicals in the correct quantities to help people digest and process the foods they consume. It has both exocrine and endocrine functions.

As an exocrine gland, the pancreas produces enzymes, such as trypsin, chymotrypsin, amylase, and lipase, which help break down food. These pancreatic juices are released into the pancreatic duct and join the common bile duct, which originates in the liver. The juices then enter the first part of the small intestine, where they begin digesting food. As an endocrine gland the pancreas e endocrine has a group of cells known as the islets of Langerhans which produce insulin and glucagon that maintain the balance of blood sugars (,,,).

METHODOLOGY

Comment [Ma5]: This should start a new paragraph.

Comment [Ma6]: What does this represents? Or put appropriate referrence

Comment [Ma7]: Please ensure font consistency

ETHICAL APPROVAL

Ethical Approval was gotten from the Research ethics committee of the Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Nnamdi Azikiwe University, Nnewi campus.

MATERIALS AND METHODS

Sixty adult Male Wistar rats, 20 pieces of pear fruits, 20 pear seeds, laboratory pipettes, a glucometer (accu check glucometer), insulin test kits, alloxan monohydrate, digital weighing balance, heparinized capillary tube, rat standard pellets meal, EDTA bottles for blood sample serum collection, metformin standard drug, hand gloves, chloroform, enclosed suitable cages, alcohol, cotton, preservative beakers, microscopes, hematoxylin and eosin, histological slides.

COLLECTION AND PREPARATION OF PLANT MATERIAL

The Fruits and seeds of avocado pear were obtained from Ekoka market, Awka, Anambra State. The fruits were cut and dried through seeverl shifting, then powdered with grinder before being sieved.

400g of the powdered fruit was soaked in 1000mL~~l~~ of distilled water for 24 hrs at temperature within room temperature with occasional and continuous shaking. It was then filtered through filter paper, and the filtrate was dried and stored in refrigerator for further use. During experiment the crude extract was distilled water diluted before the administration of extract to animals present.

CHEMICALS

Reagents used during research were analytically graded (what brands of reagent and from which country?)

3.4. MAINTENANING ANIMALS AND PROTOCOL APPROVAL

Sixty adult male Wistar rats, weighing 200–250 g were used in this study. They were housed in clean metal cages and maintained in the animal house at a 12-hour light to dark present cycles. The animals were permitted to acclimatize to condition of laboratory for one week before the administration. Standard Pellets meals were given to animals and used as thier diet during the period of the experiment. The control and experimental animals were provided with clean tap water ad libitum. The animals were maintained in accordance with the "CPCSEA guidelines for laboratory animal facility". Before, the experiment began, the animals were ~~c~~consciusly marked on the different parts of their hairy bodies, which was used as an identification mark for a particular Rat, this is to enable the response of a particular rat before and after administration.

INDUCTION OF DIABETES IN EXPERIMENTAL ANIMALS

Diabetes was induced in overnight fasted male Wistar rats by a single intraperitoneal injection of alloxan monohydrates at 200 mg/kg body weight. Blood glucose level of the rats was taken 72 hrs after alloxan administration, and diabetes was confirmed using a blood glucometer (Accu Check Sure, Taiwan). Blood samples were collected from the tip of the tail. Animals with blood glucose level equal to or more than 200 mg/dL were assigned diabetic and were used for the experiments.

Eight groups of Rats, 7 rats in each group received treatment schedules as follows

Group A: Control without alloxan treatment
Group B: Alloxan induced at 200 mg/kg of weight,(without the treatment).
Group C: Alloxan induced at 200mg/kg body weight i.p. + pericarp pear oil, (extract of fruit at the dose, 100 mg/kg of body weight);
Group D: Alloxan induced at a dose 200 mg/kg of weight of body i.p. + pericarp pear oil (extract of fruit at 200 mg/kg body weight dose)
Group E: Seed oil only (extract of fruit at 200 mg/kg dose of body weight).
Group F: 150 mg/kg alloxan induction of body weight + pericarp pear oil (fruit extract at the dose of 200 mg/kg body weight) + seed oil (fruit extract at the dose of 200 mg/kg body weight).
Group G; Pear oil + Seed oil + Control drugs metformin
Group H; Alloxan 200mg/kg induction + metformin, (standard drug).

3.9.PHYTOCHEMICAL ANALYSIS OF THE SAMPLE

Comment [Ma8]: It may be necessary to replace the word phytochemical with an appropriate word. Since what was analyzed here was blood and not plant extract

During the experiment the whole blood was used for glucose test and Plasma was used for insulin assay using Radio Immune Assay (RIA) kit for rats. Superoxide Dismutase (SOD), catalase (CAT), Glutathione Peroxide (GPx), Glutathione (GSH) and Glutathione-S-transferase (GST) were determined. After the last doses of animal treatments, animals fasted 12 hours and sacrificed by cervical dislocation. Blood samples were collected by ocular puncture before sacrifice.Serum was separated from the clot by centrifuging at 3000 rpm for 15 min. Serum check analysis of blood glucose concentration of fasted animal was measured by available glucose kit (coral clinical system, Goa, India) on basis of Trinder.

After blood collection, rats undergoed cervical dislocation for sacrifice and the pancreas from each animal harvested and fixed in 10% formal saline and used for histological examination using the H&E method.

STATISTICAL ANALYSIS

Data were presented as Mean ± Standard deviation per group. Statistical analysis was done using SPSS version 25. Analysis was done using one way analysis of variance followed by least

significant difference (LSD) test and Paired Student's t-test was done to see any difference between the paired groups. Values were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Physical and Weight observations

At the beginning of the experiment, all animals were apparently healthy and agile. During the period of inducing diabetes with alloxan animals showed signs of heavy breathing, weakness, fatigue and loss of appetite. The body of the control and experimental groups were recorded.

Rats in the Control group A had significant increase in weight. Group B that received alloxan without treatment alloxan had significant weight reductions. Group C that received alloxan 200mg/kg body weight had significantly increased body weight.

Group D and E also has significant body weight gain. Group VII; this experiment involved the combination of induced alloxan, pericarp pear oil, and seed oil. The following observations were taken, Increased level of glucose at the initiating stages causing hyperglycemia, diabetes caused destruction of pancreatic islets of Langerhans cells. But at the addition of pericarp pear oil and seed oil fruits extracts. Hypoglycemia levels were noticed. This was followed by a former decreased weight to a significant increased weights of experimental rats. This meant that regeneration of pancreatic tissues of islets of Lange hangs were observed.

Table 1: Result of changes in rat weight

Formatted: Font: 14 pt

Groups	Weights(g)	Mean ± SEM	t-Value	p-Value

A	Initial	170.08±3.74	-5.027	0.015
	Final	196 ± 0.12		
B	Initial	170.58±2.95	-3.534	0.039
	Final	143.75±12.79		
C	Initial	178.63±8.89	-3.759	0.064
	Final	218.90±6.52		
D	Initial	152.54±0.58	-1.679	0.235
	Final	198.80±6.42		
E	Initial	168.63±8.89	-3.742	0.65
	Final	218.90±6.52		
F	Initial	153.53±0.58	-3.679	0.39
	Final	199.80±26.76		
G	Initial	153.56±0.56	-3.756	0.64
	Final	200±6.53		
H	Initial	153.53±0.53	-3.759	0.235
	Final	199.90±27.79		

The result of rat weight changes presented in Table 1 shows that rats in the control group A showed that rats in the control group had a significant weight gain at the end of the experimenting period compared to the initial weight. Rats in group B however had a significant weight loss following diabetes induction compared to the initial weight. Following treatment, there were no significant weight differences for rats in groups C to H, although they all show some form of weight gain at final compared to initial.

Formatted: Font: Not Bold

Table 2 Shows Glucose Level ~~O~~of Rats ~~F~~for 4 Weeks

WEEK	**GROUPS**	MEAN+_SEM	P-VALUE
WEEK 2	A	106.667+_4.48	0
	B	350.65+_5.91	0.857
	C	261.000+_10.94	0.109
	D	251.000+_10.92	0.112
	E	93.33+_4.41	0.085
	F	106.667+_4.41	0.089
	G	262.000+_63.18	0.075
	H	212.000+_62.18	0.085
WEEK 3	A	112.3321+_4.40	0.214
	B	351.3333+_4.40	0.010
	C	119.500+_5.79	0.269
	D	260.000+_10.94	0.112
	E	251.000+_10.92	0.085
	F	241.000+_13.5332	0.089
	G	105.657+_3.3000	0.085
	H	200.651+_3.30	0.081
WEEK 4	A	119.533+_6.23	0.231
	B	360.667+_6.56	0.461
	C	173.3333+_49.69	0.174
	D	129.133+_6.43	0.798
	E	188.672+_58.86	0.269
	F	106.867+_4.40	0.269
	G	234.333+_4.40	0.797
	H	229.133+_6.43	0.785
WEEK 6	A	129.133+_6.43	0.087
	B	230.672+_58.86	0.085
	C	106.867+_4.40	0.089
	D	241.000+_13.5332	0.085
	E	105.657+_3.3000	0.085
	F	200.651+_3.30	0.089
	G	106.667+_4.41	0.075

	H	262.000+_63.18	0.798

After induction of diabetes by alloxan, diabetes was confirmed by the presence of hyperglycemia in animals and the mean level of glucose in the control group of rats was evaluated to be (range: 60–95)mg/dl, but instead, it was mg/dL (range values: 190 to 270, and) in alloxanized group. After the treatment of rats with the fruit extract of Avocado oil and it's pericarp seed oil a (100 mg/kg weight of body) the level of glucose decreased down to mg/dL h

with a range value of 156–220 mg/dL and more potential effect at 250 mg/kg dose of weight of body of fruit extract and glucose level also significantly decreased to mg/dL having range of 90–129 mg/dL.. The significant glucose concentration increase in the animals that are diabetic in comparison to that of the controlled rats is shown on the induction of alloxan.However, the oral administration of aqueous extract of Avocado fruit significantly reduced the glucose level in serum when compared with alloxan induced diabetic rats.

Effect of aqueous extract of III and IV (100 and 250 mg/kg body weight) on the serum glucose levels in alloxan induced rats. Values are the means ±S.D. for seven animals in each group. Values are significant at; statistical relevance was cchecked within groups as follows. Normal rats were compared with Diabetic rats. Iv and III groups were treated diabetic rats were compared with non diabetic rats.

HISTOPATHOLOGY STUDY

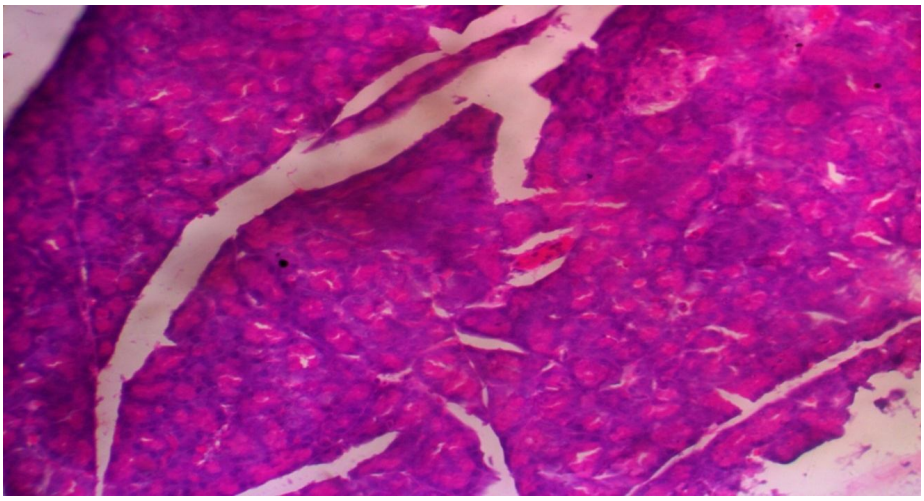


Plate 1: Which indicate normal histological features of endocrine pancreas in control group A (H&E) X 100). Photomicrograph section of pancreas shows well-spaced pancreatic acinar (PA) and Islets of Langerhans (IL) appearing normal.

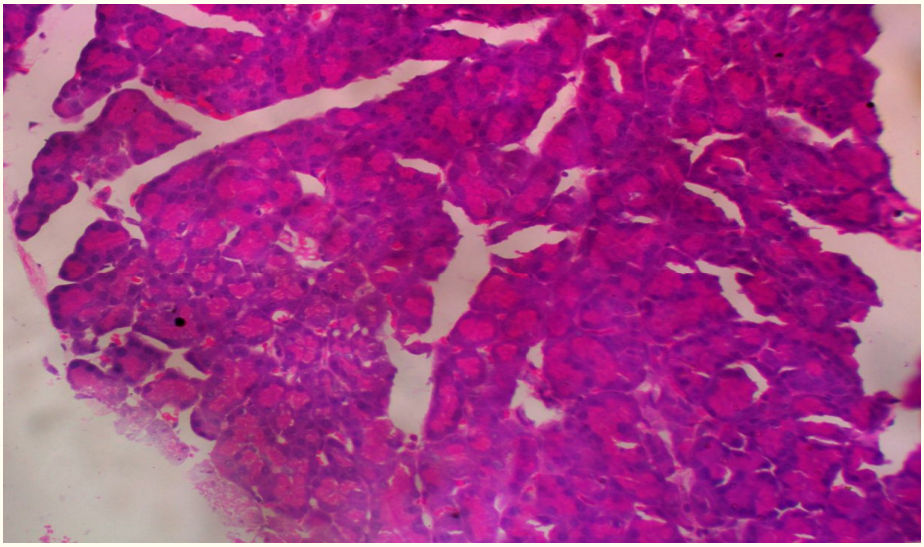


PLATE 2: Shows group B alloxan effect on the pancreatic islets of langerhans shows Photomicrograph section of pancreas degenerated pancreatic acinar (PA) and Islets of Langerhans appearing abnormal.

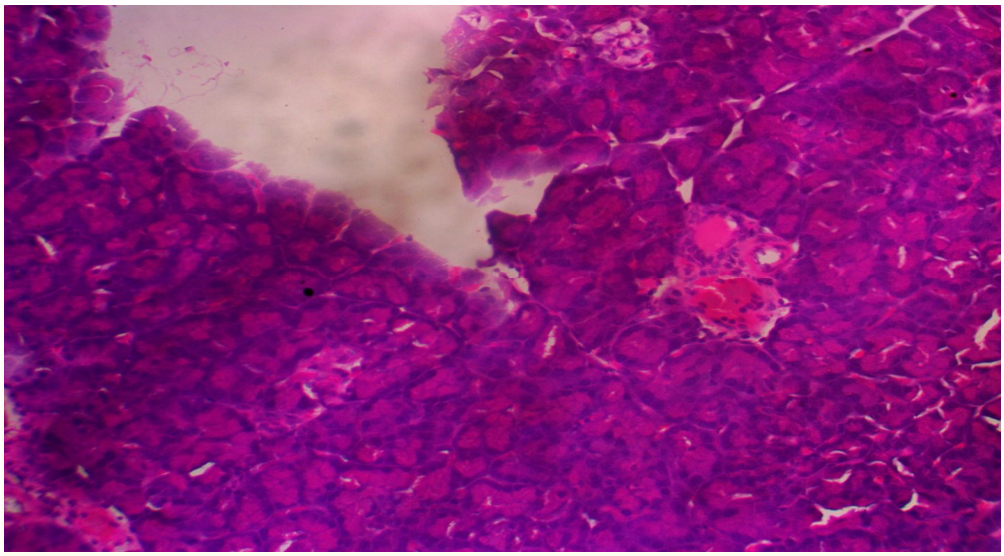


Plate 3: Which indicate normal histological features of endocrine pancreas in GROUP C and D H&E)* 100).Photomicrograph section of pancrease shows well-spaced pancreatic acinar (PA) and Islets of Langerhans appearing well prominent on treatment of Pear and Seed oil methanoic extracts.

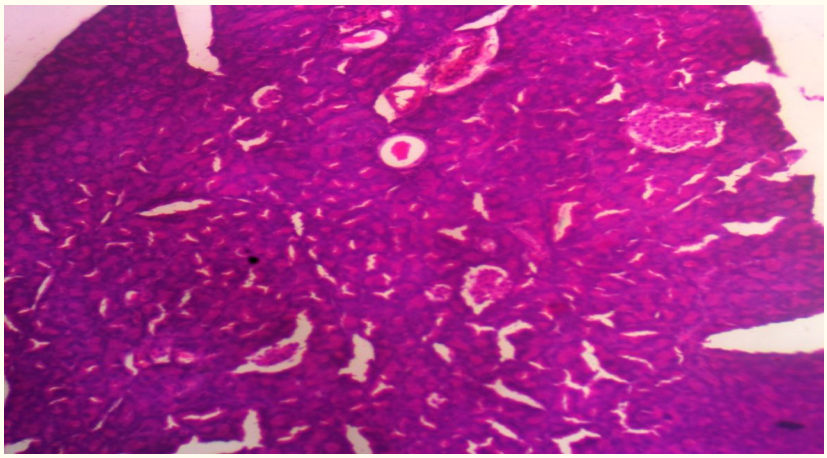
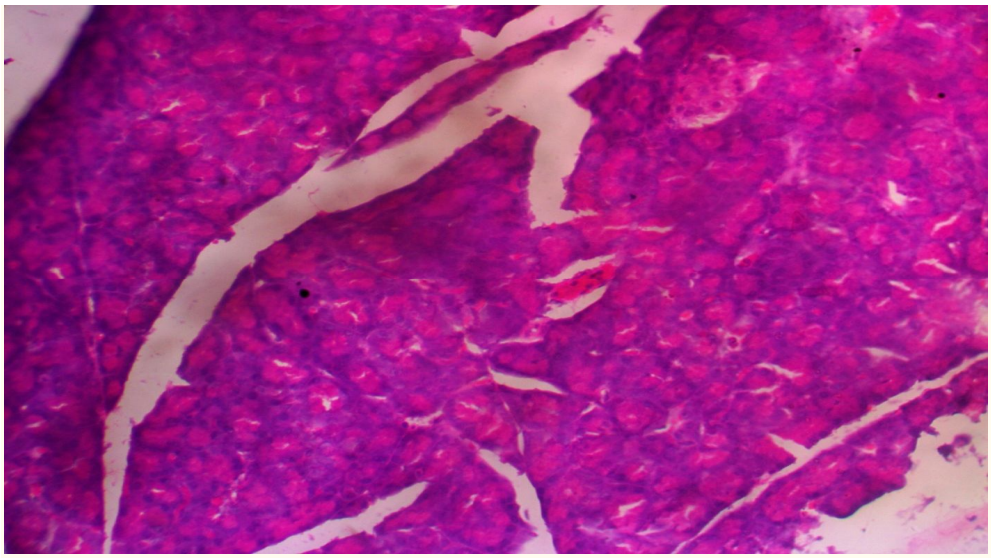


Plate 4: Which indicate slightly abnormal histological features of endocrine pancrease in control group V AND VI (H&E)* 100).Photomicrograph section of pancrease shows abnormal pancreatic acinar(PA) and Islets of Langerhans(IL) appearing a little degenerated on treatment of Alloxan ,seed and Pear oil methanoic extracts.



SLIDE 5: Which indicate slightly abnormal but rejuvenated histological features of endocrine pancrease in group VIII,(H&E)* 100).Photomicrograph section of pancreas shows abnormal pancreatic acinar (PA) and Islets of Langerhans appearing a little degenerated on treatment of Alloxan ,and Metformin standard drug methanoic extracts.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Plants that are medicinal are nature are the potential sources of bioactive agencies are being accepted worldwide. Stuudies on ethnomedicinal plants and medicinal hebals have been conducted in through time past and plants have been known for being used for pupose of medicine by tribe men in several nations. The survey on ethnobotanical can bring forth many clues for the production of drugs to cure human diseases like diabetes.(– M. Eidi, A. Midi and M. Sokhteh, “Effect of Trigonella fenugreek foenum-graecum 2020).

In Table 1, before, during and after the acclimatization windows of the rats by treatment of PERSEA AMERICANA,(Pear), seed and fruit oil methanoic extracts, and also inducing of alloxan monohydrate, and standard drugs, Metformin. Phytochemicals in Americana fruits oil includes: carbohydrates ,alkaloids, saponins, tannins, flavonoids, and resins were found in PERSEA. AMERICANA.

In table 2 there was a significant increase in Anti-oxidant levels of SOD, CAT, GPx, GST. Group,(A), Groups B, C, and D, seed oil in comparison to Groups, (B),(Alloxan group),

Group,E(Alloxan +Seed oil),Group, G, (Alloxan +Seed+ Pear Oil), Group H,(Alloxan+Metformin).

Groups, A, C, D, F, G had a relevantincrease in weight of body in comparison to Groups B,E,F at p-value and t-value $p<0.05$.

For the Glucose level, in Table 3.0, Glucose Levels were also measured for p-value, $p<0.05$ and f- value, mean and standard deviation of all groups,(A-H), were determined during 1^{st}-2^{nd} week, of acclimatization, Induction and Treatment. During the 1^{st} week of acclimatization of Albino Wister rats, Blood Glucose Level remain normal, not until the Second week and 4^{th} week. During the second- fourth week,(2-4). Mortalities were observed especially, in Groups B,(Alloxan Groups), as well as elevated Blood glucose of all groups in 3rd, 4^{th} week, blood glucose levels , had stabilize, drastically, in all groups induced and treated with alloxan monohydrate, pear oil, seed oil, and standard drug, metformin.

In confirming the levels of Insulin in all groups, in table, 4, insulin levels of group A, (control), being 6.09+_0.73, mean and standard deviation,(Evaluation, measurements), and and p-values of $P<0.05$ or $P>0.05$ were also determined .

Comment [Ma9]: Avoid the use of coma before the bracket

Although, P-value, of $P<0.05$ value for each groups (A, B, C, D, E, F, G, AND H) were observed for plasma Insulin levels of groups ,(A-H),.

Groups, B(Alloxan), Significantly, had a lower insulin value, physiologically, because of the presence of alloxan monohydrate induction, compared to Groups H, C, D,G, H, treated with pear seed, oil, and metformin,(Standard drugs).

Seed with serum normal parameters and streptozotocin- diabetic induced rats," Research Nutrition, vol. 2, 2020. Safe, effective, and cost efficient remedies are gaining grounds among the people of the urban and rural settlement s, especially in developing countries such as india.

In this study, B. L. Chaudhary,S.S Katewa and A. Jain, "Folk herbal medicine India," Journal of Ethnopharmacology,p. 41–46, 2020.PERSEA AMERICANA was selected for diabetic anti study and rejuvenation capacityof the tissues. Therefore, this study was carried out to justify a claimed use. Alloxan is the most commonly used chemical used to induce diabetes in experimental animals.

Alloxan chemical is a β-cytotoxin chemical and (S. S. Katewa, B. L. Chaudhary,pp. 41–46, 2020) induces diabetes by damaging the tissues, secreting insulin be cells in decreased insulin endogenous release. Administered Alloxan rabbits now get hyperglycemic in brief period of time, fthis is filled by glucose hepatic overproduction.

High glucose ambience can support apoptosis, by causing cellular destruction as a result of diatic hyperglycemiain.The Reactive oxygen species (ROS) are sacrosanct mediators of death of Beta cells during DM onset and development. High level of glucose has been stipulated to g

create ROS and species of nitrogen in cell types.

Superoxide Generation by high level of glucose is described and principally arise via the mitochondrial transport election chain. Another source (B. L. Chaudhary, S.S Katewa and A. Jain, no. 1, pp. 41–46, 2020) of induced glucose oxidative stress is the pathway where glucose is reduced to sorbitol by reductase aldose in a process that ejects NADPH. This will retard the NADPH-dependent gen of glutathione, an essential antioxidant cell.

In this study significant hyperglycemia was gotten after (150 mg/kg alloxan quantity if body weight) was injected. Alloxan diabetic induced rats with more than 200 mg/dL of glucose blood level were knownto be diabetic and was used for this study. However,(, B. L. Chaudhary,S.S,Katewa and A. Jain, 2020) administed the aqueous extract at the dose of 100 / 250 mg/kg of weight of body which decreased the level of glucose in alloxan induced rats.

These results are in line e with the findings of such an effect may be checked for in part by a decrease in the rats of intestinal glucose level , (, B. L. Chaudhary, pp. 41–46, 2020) absorption was achieved by an pancreatic pump action which include the stimulation of glucose.periphery utilization or glycolytic enhancement and glycogenic process with decrease in gluconeogenesis .

Effective glucose blood control is the option for preventing or reducing diabetic complications and enhancing life quality in patients with diabetes. On this basis, we have seen that glucose induces hyperglycaemic action, (S. S. Katewa, B. L. Chaudhary,2020), model to screen the antihyperglycaemic activity in plant extracts content.

The present study verified the changes in weight of body and weight of organs in control induced diabetic and treated animals for the period of the study as decrease in weight of the body is considered as a marker for diabetes development due to continuous glucose excretion and decrease in uptake periphery of glucose and synthesis of glycogen.

Our results revealed a (B. L. Chaudhary,S.S. Katewa and A. Jain, 2020), change in weight of organs and Body between induced k

alloxan and treated Rats. These results are in line with the findings that are quit contradictory by (Dans et al., 2020) who revealed Momordica charantia had no revelant effect on weight of body of a diabetic. This, (S. S. Katewa, B. L. Chaudhary, and pp. 41–46, 2020) elevates body weight of diabetic rats because of result of Persea Americana treatment may be directed to the increase in insulin release.

Results gotten from our research showed that alloxanization caused a relevant increase in uric serum acid, and decrease in albumin and protein levels (S. S. Katewa, B. L. Chaudhary, and A. Jain,2020) values gotten in animals that are diabetic when compared with those that are nondiabetic in the control.

Comment [Ma10]: Avoid using coma before bracket

Comment [Ma11]: Please remove coma

This could be due to the glycation of protein in diabetes which could lead to wasting of muscles and elevated release of purine, the original source of uric acid, as well as elevated xanthine oxidase release action.

Results of our research are consistent with the research reported by others who revealed that serum uric acid, and creatinine levels were elevated in diabetic rats. This may caused based on metabolic unrest in diabetes reflected in high level activities of lipid peroxidation, oxidase oxanthibe and elevated levels of cholesterol

Results were also reported showing the increased urea concentration and creatinine cause based on excessive lipolysis in diabetes mellitus causing ketosis and acidosis. Kidney maintains a chemical composition fluid in the body by acidification in urine and metabolic wastes removals such as urea, uric acid, and creatinine. During diseases of the renals the concentration of these metabolites elevates in blood. On the other wing treatment of extract ~~A~~aqueous ***Persea ~~A~~americana*** for 21 days on rats that are diabetic, the elevated level were now normal.

Based on this results,theserum albumin ~~decrease~~decrease and protein in animals that are diabetic were restored to rate of control by treatment of insulin, which speeds up amino acid transportation by cells and the protein manufacturing machinery of the cell.

Reduced plasma albumin was observed in induced alloxan rats which may be caused by microproteinuria and albuminuria, which is a sacrosanct clinical marker of diabetes which could also cause increased protein catabolism. Insulin lack also reduces RNA and mRNA, which is a factor for the reduction of total protein. Results also connect with findings above.

Lipid which are perooxides are Known to be secondary by products of stress oxidation and are released as a result of the effect of toxic reactive oxygen species produced in lipid during peroxidation period of diabetes.. Peroxidation of Lipids (LPO) is one of the c features of celluar chronic diabetes. Diabetes is thought that hypoinsulinemia elevates the activities of enzyme, fatty acyl coenzyme-A oxidase, which introduces beta fatty acids oxidation, resulting in LPO.

Elevated LP retards membrane activity by membrane fluidity and changing the activity of bound membrane-enzymes and receptors [60]. LPO later on result in elevated production of radicals that are free harmful to cells of the body.

Comment [Ma12]: Kindly give proper reference as it is different from others. Consistent reference format please.

However,~~,~~ peroxide Lipid mediated tissue deatruction has been examined in the development of types I and II diabetes mellitus together with insulin secretion which is close associated with lipoxygenase-derived peroxides.

Moreover, elevated LPO levels leads to cellular infiltration and islet cell destruction in diabetes. During this study, increased levels of lipid peroxidation were observed in alloxan rat treatment. There are many reports in literature that ~~E~~expresses the increased levels of lipid peroxides in the

induced alloxan diabetes rats. This normal state may be archieved by the antioxidant and radicals that are free and thier quenching nature of Persea Americana.

However, the ~~H~~hypoglycemic effect of the avocado fruit and seed extract may be due probable contents of elements such as calcium, magnesium, potassium, sodium, zinc, chromium e.t.c that play key role in blood glucose homeostasis by regulating the key enzymes involved in gluconeogenesis in the liver e.g. glucose-6- phosphatase, fruitcose-1, 6- bisphosphatatase and phosphoenolpyruvate carboxykinase, thereby blocking gluconeogenesis and enhancing glucose utilization in the body (Abdel MA, El-Feki M, 2020),The seed may in addition to these elements contains certain hypoglycemic agents such as phytochemicals (e.g. flavonoids, saponins, steroids, terpenoids, tannins and alkaloids etc) which contain insulin stimulatory substances such as insulin receptors substrate (IRS), prohornone convertase, glycogen synthase, the b3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor and peroxisome proliferators – activated receptor gamma (~~.~~Abdel MA, El-Feki M, 2020).

~~However~~It is yet unclear, the mechanism by which the extract lowered the blood glucose level in alloxan induced diabetic rats~~; is still unclear. I~~it could be by ~~(B. T. Doumas, W. Ard Watson, and H. G. Biggs, "Albumin , 2020)~~,stimulating peripheral utilization of glucose by inhibiting absorption in the gastrointestinal tract (GIT)(B. T. Doumas, W. Ard Watson, and H. G. Biggs, "Albumin , 2020), increasing glucose metabolism, or regenerating the pancreatic tissue or potentiating the insulin secretion by the surviving B- cells. A prolonged (B. T. Doumas, W. Ard Watson, and H. G. Biggs, 2020) administration of the extract shows higher hypoglycemic effects on alloxan induced diabetic rats than are shorter period. And after withdrawal of the treatment for one week the blood glucose gradually rised, however below that of the untreated group, this signifies the management effect of the avocado seed extract. The(B. T. Doumas, W. Ard Watson, and H. G. Biggs, 2020) increase in weight of diabetic rats treated with avocado seed extract (Table) was found to be significant between diabetes groups treated with avocado seed and diabetic non-treated (Group II). This could be due to certain,(B. T. Doumas, W. Ard Watson, and H. G. Biggs, 2020),compounds and or mineral elements that may stimulate effective utilization of nutrients. In addition, the seed may contain nutrients such as (B. T. Doumas, W. Ard Watson, and H. G. B, 2020) protein and fat this coupled with their effective utilization, may be responsible for the weight gain.

Comment [Ma13]: Please recast the paragraph, line 412 to line 428. It will be appropriate to have other references other than (B. T. Doumas, W. Ard Watson, and H. G. Biggs, 2020) as used in this paragraph.

CONCLUSION

Data results from the research study indicate clearly that the extract of *Persea ~~A~~americana* fruit at (100,150,200 mg/kg weight of body dose showed significant antihyperglycemic than at low dose (100 mg/kg weight of body) in the diabetic Induced rats, v biochemical parameters like KFT together with kidney tissues regeneration. Therefore, further investigations is a necessity to examine the phytoconstituent which is responsible for the anti- diabetic effect.

Formatted: Font: Italic

Comment [Ma14]: What does this V represents?

The (J. Welihinda, E. H. Karunanayake, M. H. R. Sheriff,2020,) chemical induction of diabetes appears to be the most popularly used procedure in inducing diabetes mellitus in experimental animals. The foremost drug-induced diabetic model is the alloxan diabetes that is capable of inducing type I diabetes mellitus in experimental animals. The surgical and genetic methods of diabetes induction are associated with a high percentage of animal morbidity and mortality. Hence, alloxan induceddiabetes model appears to be the most reliable and easily reproducible method of inducing diabetesmellitus in experimental animals. So, efforts should be made towards (J. Welihinda, E. H. Karunanayake, M. H. R. Sheriff, and K. S. A. Jaya, 2020), upbringing and uplifting the model of alloxan induced diabetes mellitus in the experimental animals.

Eventually,the plant extract exerted a dose-dependent protective effect on the pancreas, kidneys and liver, like the reference drug Metformin.(B. T. Doumas, W. Ard Watson, and H. G. Biggs,2020)Taken together, the results of present study provide a pharmacological basis for the folkloric use of the hot-water extract of *Persea. Americana* seeds and Pericarp Pear oil in the management of diabetes mellitus.

Comment [Ma15]: Please remove full stop before bracket opening.

Furthermore, this results show that combination of this seeds and fruits can form good dietary combination of Healthy Meals and Ready to Use Therapeutic Foods. Which could be produced for both Adult and Young Ones to improve their Health States.

RECOMMENDATIONS

I recommend that extensive researches be carried out with other fruit extracts and standard drugs.

REFERENCE

A. Aloulou, et al., “Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats,” BMC Complementary and Alternative Medicine, vol. 12,,article 63,2020: pp.235-345.

A. Bener, et al “Genetics, obesity, and environmental risk factors associated with type 2 diabetes,” Croatian Medical Journal, vol. 46, no. 2, 2020; pp. 302–307, .

A. C. Guyton and J. E. Hall, Textbook of Medical Physiology, WB Saundersp, Philadelphia, Pa, USA, 10th edition, 2020:pp. 220-300,

A. Wifi et al, “Effect of fenugreek (Trigonella foenum-graecum L.) seeds on serum parameters in normal and streptozotocin-induced diabetic rats,” Nutrition Research, vol. 27, no. 11, 2020, pp. 728–733,

A. Kalafi et al, “Anti-hyperglycemic and antioxidant properties of Cassia auriculata leaves and flowers on alloxan induced diabetic rats,” Pharmacologyonline, vol. 1, 2020,pp. 204–217.

A. Datin et al., “The effect of Momordica charantia capsule preparation on glycemic control in Type 2 Diabetes Mellitus needs further studies,” Journal of Clinical Epidemiology, vol. 60, no. 6, 2020, pp. 554–559

A.Jayasooriya,, et al“Effects of Momordica charantia powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol- enriched diets,” Journal of Ethnopharmacology, vol. 72, no. 1-2,2020, pp. 331–336.

A. Paul et al “Medicinal uses and molecular identification of two Momordica charantia varieties - a review,” Electronic Journal of Biology, vol. 6, no. 2, 2020,pp. 43–51.

Abdel et al Effect of Nigella sativa, fish oil and gliclazide on alloxan diabetic rats. Biochemical and Histopathological studies. *J Egy Ger Soci Zool.2020: 237–265*

Adewole et al. Insulin-induced immunohistochemical and morphological changes in pancreatic β-cells of streptozotocin-treated diabetic rats. *Methods Find in Exp Clin Pharmacol.2020* :447–455.

Alhassan AJ, et al . Effects of aqueous avocado pear (Persea americana) seed extract on alloxan induced diabetes rats. *Greener J Med Sci.*:2(1)2020: 5–11.

Antia BS et al Hypoglycemic activity of aqueous leaf extract of Persea americana Mill. *Indian J Pharmacol.*)2020:325–326.

B. A. Salau, et al “Effects of fruits and vegetables on cardiovascular disease risk factors in non-insulin dependent diabetes mellitus (NIDDM) subjects,” African Journal of Medical and Pharmaceutical Sciences, vol. 7, 2020: pp. 21–26.

B. T. Doumas, et Al “Albumin standards and the measurement of serum albumin with bromcresol green,” ClinicaChimica Acta, vol. 31, no. ,(2020): pp. 87–96.

Bonilla JV, et al. Methods and Composition for regulation of blood cholesterol. *Afri J Traditional Complement Altern Med.* 2020;6(4):573–578.

D. Allen, et al, “High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases,” The FASEB Journal, vol. 17, no. 8, (2020),pp. 908–910

D. Kleiner, et al., “Design and validation of a histological scoring system for nonalcoholic fatty liver disease,” Hepatology, vol. 41, no. 6, (2020),pp. 1313–1321

D. Lorke, “A new approach to practical acute toxicity testing,” Archives of Toxicology, vol. 54, no. 4, (2020),pp. 275–287

501 D. Sathishsekar et al “Antioxidant properties of Momordica Charantia (bitter gourd) seeds on
502 Streptozotocin induced diabetic rats,” Asia Pacific Journal of Clinical Nutrition, vol. 14, no. 2,
503 (2020),pp. 153–158,

504 Dunn JS, et al. Necrosis of islets of Langerhans produced experimentally. Lancet 1943;1;484-
505 7.2020. Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the
506 Rat Pancreas Physiol Res; (2020),50:536-46.

507 E. H. Karunanayake et al, “Search of novel hypoglycaemic agents from medicinal plants,” in
508 Diabetes Mellitus and Its Complications-An Update, A. K. Sharma, Ed., (2020), pp. 192–196,
509 Macmillan, India,

510 Edem et al Effect of aqueous extracts of alligator pear seed (Persea americana Mill) on blood
511 glucose and histopathology of pancreas in alloxan-induced diabetic rats. *Pak J Pharm Sci.*;22(3)
512 (2020),:272–276.

513 Edem et al Hypoglycemic Effects of Ethanolic Extracts of Alligator Pear Seed (Persea
514 Americana Mill) in Rats. *Euro J Sci Res* ;33(4), (2020),:669–678.

515 Fabiyi AK, et al. The impact of knowledge, attitude, practice and belief of type two Nigerian
516 diabetes patients on drug compliance. *Diabetes Int*;12(1) (2020),:15–17.

517 G. L. Bakrik et Al, “Diabetic nephropathy: What you need to know to preserve kidney function,”
518 Postgraduate Medicine, vol. 93, no. 5, (2020),pp. 89–93

519 Gray AM, et Al, Insulin–releasing and insulin–like activity of the traditional anti-diabetic plant
520 Coriander sativum (coriander) *Br J Nutr.* 2020, ;81(3) (2020):208–209.

521

522 H. Ohkawa et al,“Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction,”
523 Analytical Biochemistry, vol. 95, no. 2, H. Hwang, et al “Role of reactive oxygen species in the
524 pa2.1 Autoimmune Mediated Islets Cells , (2020),pp. 351–358

525 H. Van Herck, et al., “Orbital sinus blood sampling in rats as performed by different animal
526 technicians: the influence of technique and expertise,” Laboratory Animals, vol. 32, no. 4,
527 (2020),pp. 377–386

528 H.-L. Cheng, et al “A cell-based screening identifies compounds from the stem of Momordica
529 charantia that overcome insulin resistance and activate AMP-activated protein kinase,” Journal
530 of Agricultural and Food Chemistry, vol. 56, no. 16, (2020),pp. 6835–6843

531 Harborne JB. London (UK): "Chapman and Hall Ltd; 1973. Phytochemical Methods: A guide to
532 modern techniques of plant analysis; –49.(2020),pp. 188

I. G. Obrosova, et al., “Aldose reductase inhibitor fidarestat prevents retinal oxidative stress and vascular endothelial growth factor overexpression in streptozotocin-diabetic rats,” Diabetes, vol. 52, no. 3,(2019), pp. 864–871,

Iloh G, et al. "Obesity in adult in Nigeria: A study of its pattern and common primary co-morbidities in a rural Mission General Hospital in Imo state, south-eastern Nigeria. *Niger J Clin Pract.* 2020;14(2)(2020):212–2020.

Imafidon KE, et al. "Effects of Aqueous Seed Extract of Persea Americana Mill. (Avocado) on Blood Pressure and Lipid Profile in Hypertensive Rats. *Advan Biol Res*;4(2)(2020):116–112.

J. Item JA,Histological Effect of Combined Extracts of Vernoniaamygdalina and AzadirachtaIndica on Normal and Diabetic Rats: the Pancreas and Liver. *Res J Agr Biol Sci.*6(4)(2020):514–523.

J. C. Will, et al “Is diabetes mellitus associated with prostate cancer incidence and survival?” Epidemiology, vol. 10, no. 3, (2020):pp. 313–318

J. K. Fawcett et al, “A rapid and precise method for the determination of urea,” Journal of Clinical Pathology, vol. 13,(2020): pp. 156–159

J. L. Evans, et al “Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes,” Endocrine Reviews, vol. 23, no. 5, (2020):pp.599–622

J. Pitipanapong, et al“New approach for extraction of charantin from Momordica charantia with pressurized liquid extraction,” Separation and Purification Technology, vol. 52, no. 3, (2020):pp. 416–422

J. V. Hunt, et al, “Oxidative alterations in the experimental glycation model of diabetes mellitus are due to protein-glucose adduct oxidation: some fundamental differences in proposed mechanisms of glucose oxidation and oxidant production,” Biochemical Journal, vol. 291, no. 2, (2020):pp. 529–535

J. Virdi, et al “Antihyperglycemic effects of three extracts from Momordica charantia,” Journal of Ethnopharmacology, vol. 88, no. 1, (2020): pp. 107–111

J. Welihinda,et al, “Effect of Momordica charantia on the glucose tolerance in maturity onset diabetes,” Journal of Ethnopharmacology, vol. 17, no. 3,(2020): pp. 277–282

João JJGL, et al. "Chemical composition, toxicity and larvicidal and antifungal activities of Persea americana (avocado) seed extracts. *Revista da SociedadeBrasileira de Medicina Tropical.* ;42(2)(2020):110–150.

K. Yue,et al., “Alterations in antioxidant enzyme activities in the eyes, aorta and kidneys of diabetic rats relevant to the onset of oxidative stress,” Life Sciences, vol. 77, no. 7,(2020): pp. 721–734

K. Yue,et al “Redox changes precede the occurrence of oxidative stress in eyes and aorta, but not in kidneys of diabetic rats,” Life Sciences, vol. 73, no. 20, (2020):pp. 2557–2570

K. Rajagopal et al, “Antihyperglycaemic and antihyperlipidaemic effects of Nymphaea stellata in alloxan-induced diabetic rats,” Singapore Medical Journal, vol. 49, no. 2, (2020):pp. 137–14.

L. Ruiz-Muñoz, et al, “Enalaprilat inhibits hydrogen peroxide production by murine mesangial cells exposed to high glucose concentrations,” Nephrology Dialysis Transplantation, vol. 12, no. 3, (2020):pp. 456–464

Lukačínová A et al. Ništiar: "Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. *Acta Vet Brno.* 2020;77(2):175–182.

M. Anjane et al “Effect of irbesartan on the antioxidant defence system and nitric oxide release in diabetic rat kidney,” American Journal of Nephrology, vol. 24, no. 5, (2020): pp. 488–496

M. G. Moghaddam,et al, “The Effects of Origanum Majorana on oxidative stress and histopathology of renal tissue among streptozotocin-induced diabetic rat,” Thrita Journal of Medical Sciences, vol. 2, no. 1, (2020):pp. 29–34

M. M. Dallak,et al., “Oxidative stress as a common mediator for apoptosis induced-cardiac damage in diabetic rats,” The Open Cardiovascular Medicine Journal, vol. 2, 2020;pp. 70–78

Mahadeva RUS, et al "Insulin Stimulative and Anti-Oxidative Effects of Persea americana Fruit Extract on Streptozotocin Induced Hyperglycemic Rats. *J Med Biol Sci.* 2020;4(1):1–10.

N. Hfaiedh,et Al “Diabetes-induced damages in rat kidney and brain and protective effects of natural antioxidants,” Journal of Nutrition & Food Sciences, vol. 3, (2020): no. 4

N. Makare, et al “Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice,” Journal of Ethnopharmacology, vol. 78, no. 2-3, (2020): pp. 133–137

N. Orsolic et al “Honey bee products and their polyphenolic compounds in treatment of diabetes,” in Phytopharmacology and Therapetutic Values IV, J. N. Govil and V. K. Singh, Eds., vol. 22, (2020): pp. 455–471, Stadium Press

N. Oršolić et al, G. Gajski, V. Garaj-Vrhovac, D. Crossed D Signikić, Z. Š. Prskalo, and D. Sirovina, “DNA-protective effects of quercetin or naringenin in alloxan-induced diabetic mice,” European Journal of Pharmacology, vol. 656, no. 1–3, (2020):pp. 110–118, 2020

595 N. Fernandes,et al “An experimental evaluation of the antidiabetic and antilipidemic properties
596 of a standardized Momordica charantia fruit extract,” BMC Complementary and Alternative
597 Medicine, vol. 7, article 29, 2020,pp. 133–137

598 N. Wadood,et al, “Effect of Ficus relegiosa on blood glucose and total lipid levels of normal and
599 alloxan diabetic rabbits,” Journal of Ayub Medical College, Abbottabad, vol. 15, no. 4,
600 (2020):pp. 40–42

601 N. Wadood, et al, “Effect of a compound recipe (medicinal plants) on serum insulin levels of
602 alloxan induced diabetic rabbits,” Journal of Ayub Medical College, Abbottabad, vol. 19, no. 1,
603 (2020):pp. 32–38

604 Nakashima N, et al "Isolation of pseudoprototimosaponin AIII from rhizomes of
605 anemarrhenaasphodeloides and its hypoglycemic activity in steptozotocin-induced diabetic
606 mice. *J Nat Prod.56*(30) (2020):345–350.

607 N'guessan et al. " Effect of Aqueous Extract of Persea Americana Seeds on the Glycemia of
608 Diabetic Rabbits. *Eur j Sci Res.*26(3) (2020):376–385.

609 Nyenwe AE,et al "Type II diabetes in adult Nigerians: A study of its prevalence and risk
610 factors. *Diabet Res Clin Pract.*62(3) (2020):177–188.

611 O. Lowry, et al “Protein measurement with the Folin phenol reagent,” The Journal of Biological
612 Chemistry, vol. 193, no. 1, (2020):pp. 265–275

613 Ogbera AO,et al. "The foot at risk in Nigerians with diabetes mellitus: The Nigerian
614 scenario. *International J EndocrMetab.* (2020) ;4:165–173.

615 Okonta M,et al. "Blood Glucose Lowering Activities of Seed of Persea americana On Alloxan
616 Induced Diabetic Rats. *Nig J Nat Prod and Med.* (2020) ;11:26–28.

617 P. Chaturvedi,et al, “Effect of Momordica charantia on lipid profile and oral glucose tolerance in
618 diabetic rats,” Phytotherapy Research, vol. 18, no. 11, (2020):pp. 954–956.

619 P. Mayne,et al “Lipid metabolism,” in Clinical Chemistry in Diagnosis and Treatment, pp. 240–
620 243, Clays publisher, London, UK, 6th edition,vol. 8, no. 5, (2020):pp. 700–720

621 P. Fossati,et Al “Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone
622 chromogenic system in direct enzymic assay of uric acid in serum and urine,” Clinical
623 Chemistry, vol. 26, no. 2, vol. 18,(2020);pp. 227–231.

624 P. Luzi, et al, “Characterization of the rhesus monkey galactocerebrosidase (GALC) cDNA and
625 gene and identification of the mutation causing globoid cell leukodystrophy (Krabbe disease) in
626 this primate,” Genomics, vol. 42, no. 2,(2020); pp. 319–324.

627 P. Maisonneuve et al., “Cancer in patients on dialysis for end-stage renal disease: an
628 international collaborative study,” The Lancet, vol. 354, no. 9173,(2020); pp. 93–99.

629 P. Trinder et al “Determination of glucose in blood using glucoseoxidase with an alternative
630 oxygen acceptor,” Annals of Clinical Biochemistry, vol. 6, (2020);pp. 24–27.

631 R. Rahimi, “A review on the role of antioxidants in the management of diabetes and its
632 complications,” Biomedicine and Pharmacotherapy, vol. 59, no. 7, (2020);pp. 365–373.

633 R. Bonsnes “On the colorimetric determination of Creatinine by the Jaffe reaction,” The Journal
634 of Biological Chemistry, vol. 158, (2020);pp. 581–591.

635 S. Ahmed, et Al “Comparative efficacy of neem and karela with insulin and glibenclamide on
636 biochemical parameters in rabbit,” Journal of Animal and Veterinary Advances, vol. 4, no. 2,
637 (2020);pp. 221–223.

638 S.Rashmi et al, “Antidiabetic effect of Euphorbia hirta leaves in alloxan induced diabetic mice,”
639 Pharmacologyonline, vol. 1, (2020);pp. 61–69.

640 S.Lenzen,“The mechanisms of alloxan- and streptozotocin-induced diabetes,” Diabetologia, vol.
641 51, no. 2, (2020);pp. 216–226.

642 S. Chung et al, “Contribution of poloyl pathway to diabetes-induced oxidative stress,” Journal of
643 the American Society of Nephrology, vol. 14, no. 8, (2020);pp. 233–236.

644 S.Katewa,et al, “Folk herbal medicines from tribal area of Rajasthan, India,” Journal of
645 Ethnopharmacology, vol. 92, no. 1, (2020);pp. 41–46.

646 Shanmugasundara et al "Possible regeneration of the islets of Langerhans in streptozocin diabetic
647 rats given Gymnemasylvester leaf extracts. *J Ethnopharmcol.* 2020:pp.265–269.

648 Sievenpiper JL, et al "Null and Opposing Effects of Asian Ginseng (Panax ginseng C A Meyer)
649 on Acute Glycaemia: Result of Two Acute Dose Escalation Studies. *J Am Coll*
650 *Nutr*;22(6)(2020);pp.524–532.

651 Singh SN, et al, "Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in
652 streptozotocin induced diabetic rats. *J Ethnopharmcol.73*(3)(2020);pp.269–277.

653 Sogbade AO.et al, "Anti-diabetic and antioxidant effects of Zingiber Officinale on alloxan-
654 induced and insulin-resistant diabetic male rats. Niger J Physiol Sci 2020;26:89-96.

655 T. Almdal et al “Strict insulin therapy normalises organ nitrogen contents and the capacity of
656 urea nitrogen synthesis in experimental diabetes in rats,” Diabetologia, vol. 31, no. 2, (2020);pp.
657 114–122.

T. Szkudelski, et al “The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas,” Physiological Research, vol. 50, no. 6, (2020);pp. 537–546.

T. Tuvemo, et al, “Serum magnesium and protein concentrations during the first five years of insulin-dependent diabetes in children,” Acta Paediatrica, International Journal of Paediatrics, Supplement, vol. 86, no. 418, (2020);pp. 7–10.

Teoh SL,et al "Histological changes in the kidneys of experimental diabetic rats fed with Momordica charantia (bitter gourd) extract. *Romanian J Morphol Embryo*;51(1)(2020);pp.91–958.

Tiaka D.et al." Diabetic mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr Sci.* 2020;83(1):30–37.

W. Winters,et al“Inhibition of the progression of type 2 diabetes in the C57BL/6J mouse model by an anti-diabetes herbal formula,” Phytotherapy Research, vol. 17, no. 6, (2020);pp. 591–598.

Wokoma Fs "Diabetes and hypertension in Africa- An overview. *Diabetes Int*;12(12):(2020);pp.36–40.

X.-F. Zhang et al, “Antihyperglycaemic and anti-oxidant properties of Andrographis paniculata in normal and diabetic rats,” Clinical and Experimental Pharmacology and Physiology, vol. 27, no. 5-6, (2020);pp. 358–363.

X.-Q. Yuan, et al “Hypoglycemic effect of semipurified peptides from Momordica charantia L. var. Abbreviata Ser. in alloxan-induced diabetic mice,” Journal of Food Biochemistry, vol. 32, no. 1, (2020);pp. 107–121.

Y. Y. Soon and B. K. H. Tan, “Evaluation of the hypoglycemic and anti-oxidant activities of Morinda officinalis in streptozotocin-induced diabetic rats,” Singapore Medical Journal, vol. 43, no. 2, (2020);pp. 77–85.

688

689

690

691