WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 7, 1384-1407. Research Article ISSN 2277–7105

EFFECT OF METHANOLIC SEED EXTRACT OF *MANIHOT* ESCULENTA ON THE TESTES OF ALLOXAN-INDUCED DIABETIC MALE WISTAR RATS

Ikueze Ijeoma Chiamaka*, Dr. Ofoego Uzozie Chikere and Muorah Chinecherem Onyekachi

Department of Human Anatomy, Nnamdi Azikwe University, Nnewi, Anambra State.

Article Received on
21 February 2024,

Revised on 11 March 2024,
Accepted on 31 March 2024

DOI: 10.20959/wjpr20247-31906

***Corresponding Author**
Ikueze Ijeoma Chiamaka
Department of Human Anatomy, Nnamdi Azikwe University, Nnewi, Anambra State.

ABSTRACT

Diabetes mellitus is a chronic disorder of glucose metabolism with serious clinical consequences (Nita and Nicholas, 2010). Type 2 diabetes mellitus (T2DM) is a chronic complex condition associated with hyperglycaemia and long-term end organ damage. DM induces many functional and structural syndromes and complications in multiple organs, such as testis, brain, heart and retina (Lingli et al, 2018). Several studies have been made on the impact of diabetes on organ functions. Over time it became clear that death and disability from diabetes complications involving the eyes, kidneys, peripheral nerves, heart, and vasculature could occur even with treatment (Elizabeth and MD, 2010). Type 2 diabetes mellitus (T2DM) can cause testicular damage which induces male infertility as a result of reduced male testicular function (Lingli Long et al, 2018). Another research shows that in human with diabetes, spermatogenesis, sperm count, sperm motility, seminal fluid volume and testosterone levels are lower compared to healthy individuals (Sexton and Jarow, 1997). Further studies have shown that Diabetics had reduced testicular volume, semen volume, and total motile sperm output while plasma LH and FSH levels were elevated. Reduction in semen volume and impotence were more common in long-standing complicated diabetes (Handelsman, Conway, Boylan, Yue and Turtle, 1985). Several researches has been put in place to discover the effect of diabetes on several organ but much work has not been done regarding it's effect in the testes. There is also a need to discover if the organic extract from *Manihot* esculenta can serve as remedy for the reduction in testicular function. A total of forty-five (45) male Wister rats weighing between 120-150g

were used for this study. They were acclimatized for 2 weeks before the commencement of the study. After acclimatization, the animals were grouped into eight groups (A, B, C, D, E, F, G and H) of between four to nine rats in a group. Group A the control group was given only normal saline. Group B was given only Cassava seed extract low, Group C was given high dose of Cassava seed extract, Group D was induced with Alloxan alone, Group E received Alloxan + low dose of cassava seed extract, Group F received Alloxan + high dose cassava seed extract, Group G received Cassava seed extract high + alloxan and finally Group 8, also received alloxan and was Treated with metformin. Results showed a reduction in the body weight as shown by the group D, this implying that alloxan monohydrate impacts on the weight of the organ thereby causing its reduction. . Group E which was induced with Alloxan monohydrate and then treated with *Manihot esculenta* showed a non significant increase in weight. 1000mg/kg of the extract when taken before inducing alloxan shows a highly significant increase in weight, signifying that the extract possesses ameliorative properties. There was also noticeable reduction in FSH,LH and testosterone Level when induced with alloxan. There was also a reduction in Sperm motility, sperm count and sperm activity.

KEYWORDS: *Manihot*esculenta, Diabetic testicular damage, Alloxan-induced diabetes, Testicular tissue regeneration.

INTRODUCTION

Diabetes mellitus is a chronic disorder of glucose metabolism with serious clinical consequences (Nita and Nicholas, 2010). Type 2 diabetes mellitus (T2DM) is a chronic complex condition associated with hyperglycaemia and long-term end organ damage. This long-term end organ damage due to exposure to hyperglycaemia leads to increased risk of cardiovascular disease and excess mortality (Ram, Bala and Melanie, 2010). DM induces many functional and structural syndromes and complications in multiple organs, such as testis, brain, heart and retina (Lingli et al, 2018).

Several studies have been made on the impact of diabetes on organ functions. Over time it became clear that death and disability from diabetes complications involving the eyes, kidneys, peripheral nerves, heart, and vasculature could occur even with treatment (Elizabeth and MD, 2010).

The testis is the male reproductive gland that is responsible for producing sperm and making androgens. They are oval shaped reproductive structures found in the scrotum. They are male

sex glands with endocrine and exocrine functions. The spermatic cord suspends the superior aspect of the testes. At the inferior end, the testes are attached to the scrotum by the scrotal ligament which is a remnant of the gubernaculum (Tiwana and Leslie, 2022).

Type 2 diabetes mellitus (T2DM) can cause testicular damage which induces male infertility as a result of reduced male testicular function (Lingli Long et al, 2018). Another research shows that in human with diabetes, spermatogenesis, sperm count, sperm motility, seminal fluid volume and testosterone levels are lower compared to healthy individuals (Sexton and Jarow, 1997).

Further studies have shown that Diabetics had reduced testicular volume, semen volume, total and total motile sperm output while plasma LH and FSH levels were elevated. Reduction in semen volume and impotence were more common in long-standing complicated diabetes (Handelsman, Conway, Boylan, Yue and Turtle, 1985).

Alloxan, chemically known as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog (Lenzen, 2008). Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti-insulin serum; alloxan and streptozotocin (STZ) are the most widely used in diabetes studies (Osasenaga, Abiola and Oluseyi, 2017).

Cassava (*Manihot*esculentaCrantz) is an important food security crop in many parts of the developing world. The crop's high yield potential and multitude of uses–both for nutrition and processing–render cassava a promising driver for the development of rural value chains (de Oliveira EJ et al, 2020). Cassava plants produce small seeds in capsules. At maturity, the capsules which are similar in shape and size to those of Castor oil (Ricinuscommunis) seeds turn blackish and explode to release the tiny seeds. Seeds of most plants are known to be rich in oils and fats. However, there are very scanty reports on the properties of oil from cassava seeds as most attention has been focused on utilization of the roots and the leaves for human consumption and livestock feeds. Apart from a couple of domestic related uses, local communities in Nigeria use oil extracted from cassava seeds for the treatment of infections

caused by opportunistic skin pathogenic microorganisms such as Staphylococcus aureus, Propionibacterium acnes, Piiyrosporumovale and Candida albicans.(Popoola et al, 2006).

Procurement of Experimental Animals

A total of forty-five (45) male Wister rats weighing between 120-150g obtained from the Animal House of the College of Health Sciences and Technology, was used for this study.

They were acclimatized for 2 weeks before the commencement of the study.

3.1.5 Procurement and identification of plant material Cassava seed was procured from local cassava farms in Amesi, Anambra state, and was identified in the Botany department of NnamdiAzikiwe University.

3.1.6 Housing of Experimental Animals

They were housed in well-aerated laboratory cages, under room temperature and 12hr light and 12hr dark cycle in the animal house of the Department of Anatomy NnamdiAzikiwe University. They were fed with standard rat feed and distilled water. All experimental procedures complies with the commendations provided in the "Guide for the care and use of laboratory Animals" prepared by The National Academy of Sciences and published by the National Institute of Health (1985).

3.2 Methods Deployed

3.2.1 Preparation of plant extract

Fresh cassava seed were harvested from a local farm in Amesi, Aguata LGA of Anambra state.

To get this seed, the pods that grow on the cassava plant were gotten and dried. While drying, the pods were allowed to pop by itself and then the seeds was removed from them. These seeds were then dried under ambient temperature after separating the pods from the seed.

The dried cassava seed were grounded using a local grinder into a coarse form. 250g of the seed were soaked in 100mls of absolute BOH England for 48 hours after which the mixture were sieved using a porcelain cloth and were further filtered using Whatman no 1 filter paper into a clean glass beaker. The filtrate was concentrated using a digital rotary evaporator (TT – 52Techmel and Techmel USA) and was further dried using a thermostat oven and stored in a refrigerator.

Experimental Design

After acclimatization, the animals were grouped into eight groups (A, B, C, D, E, F, G and H) of between four to nine rats in a group.

Group 1: Control.

Group 2: Cassava seed extract low

Group 3: Cassava seed extract high

Group 4: Alloxan alone

Group 5: Alloxan + cassava seed extract low

Group 6: Alloxan + cassava seed extract low

Group 7: Cassava seed extract high + alloxan

Group 8: Treatment with metformin.

Induction of diabetes mellitus

Induction of diabetes was done intra peritoneally according to Farshid et al (2015). The animals were fasted for 12-16 hours prior to the experiment to prevent hypoglycemia. Alloxan monohydrate (Sigma-Aldrich, USA) was dissolved in sterile saline to obtain a concentration.

The solution was injected into the peritoneal cavity of rats using a 26-gauge needle and a 1 mL syringe. The injection was given slowly to avoid any injury to the organs. The animals were then monitored for any adverse reactions such as breathing difficulties, bleeding or swelling at the injection site, or changes in behavior. After injection, the animals were provided with food and water ad libitum. Blood glucose levels was measured after 24 hours, and animals with blood glucose levels greater than 250 mg/dL were considered diabetic.

3.10 Termination of experiment

At the end of the 21 days, animals were anesthetized with chloroform in an enclosed container for 24 hours. Then animals were sacrificed, blood collected through ocular puncture, centrifuged for biochemical analysis while the organs were harvested, and fixed in 10% normal formalin for histological studies.

Determination of body weight of Animals

The body weight of the experimental animals in all groups were measured twice weekly using the animal weighing balance.

Histological study

Tissues (Brain and Testes) were fixed in 10% formol saline and were dehydrated in four (4) concentrations of absolute ethanol, i.e. 70%, 80%, 90%, 100% for 1hour each and then cleared in xylene before embedding in molten paraffin wax to remove the absolute ethanol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues were stained using.

Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues sectioned were examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (Ahmed, 2016).

STATISTICAL ANALYSIS

RESULTS

Table 4.3: Effect of methanolic seed extract of *Maniholtesculenta* on body weight in alloxan diabetic rats.

	Initial weight (g)	Final weight (g)	P-value	T-value
	MEAN±SEM	MEAN±SEM		
Group A (control)	154.33±4.70	227.33±2.19	0.001**	-27.591
Group B (100 mg/kg of MSME)	156.00±7.64	195.67±9.13	0.002**	-21.373
Group C (1000 mg/kg of MSME)	159.67±4.70	205.33±3.93	0.024**	-6.286
Group D (120mg/kg of AMH)	159.67±4.70	137.33±1.45	0.005 **	3.716
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	139.33±10.84	158.00±15.95	0.085 [a]	-3.212
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	148.00±4.04	165.67±6.33	0.049**	-4.357
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	151.00±7.00	204.00±9.5.29	0.044**	-4.596
Group H (120mg/kg of AMH + 500 mg/kg of MET)	153.67±4,48	175.00±7.00	0.015**	-8.194

Data was analysed using T-paired test and values were considered significant at *p<0.05*. SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of*Maniholtesculenta*, **: significant, [a]: not significant.

Table 4.3 result showed a significant increase in the body weight in groups A, B, C, F, G, and H while group D had a significant decrease and group E had a non-significant increase when the initial weight was compared to the final weight.

Table 4.4: Effect of methanolic seed extract of *Maniholtesculenta* on the relative Testes weight in alloxan diabetic rats.

	Relative Testes weight (g)
	MEAN±SEM
Group A (control)	0.54±0.012[b, c]
Group B (100 mg/kg of MSME)	0.81±0.09[a, b, c]
Group C (1000 mg/kg of MSME)	0.75±0.02[a, b, c]
Group D (120mg/kg of AMH)	0.78±0.06[a, c]
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	0.57±0.17[, b, c]
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	0.63±0.01[a, b, c]
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	0.67±0.00[a, b, c]
Group H (120mg/kg of AMH + 500 mg/kg of MET)	0.56±0.16[a, b]
F-Ratio	1.24

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*.

SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihotesculenta*, [*]: significant when compared with group A (control group), [a]: not significant when compared with group A(control group), [#]: significant when compared with group D(diabetic group), [b]: not significant when compared with group D(diabetic group), [@]: significant when compared with group H(metformin group), [c]: not significant when compared with group D(metformin group).

The mean relative Brain weight and the mean relative Testes weight of the study were shown on table 4.4.

Relative Testes weight

Analysis showed that there was no statistically significant difference when compared with groups A (control), D (diabetic), and H (metformin).

Table 4.6 Effect of methanolic seed extract of *Maniholtesculenta* on FSH level, LH level and Testosterone in alloxan diabetic rats.

	FSH (mlU/ml)	LH (mlU/ml)	TESTOSTERONE(ng/ml)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	70.00±5.77$^{\#, @}$	64.66±1.45$^{\#, @}$	1.36±0.08$^{\#, @}$
Group B (100 mg/kg of MSME)	33.66±3.17$^{*, b, c}$	19.33±0.88$^{*, \#, @}$	0.30±0.05$^{*, b, c}$
Group C (1000 mg/kg of MSME)	33.33±3.33$^{*, b, c}$	26.00±0.57$^{*, \#, c}$	0.50±0.05$^{*, b, c}$
Group D (120mg/kg of AMH)	43.33±6.66$^{*, @}$	42.00±3.05$^{*, @}$	0.40±0.15$^{*, c}$
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	63.33±3.33$^{a, \#, @}$	48.00±0.57$^{*, \#, @}$	0.70±0.05$^{*, \#, c}$
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	70.00±5.77$^{a, \#, @}$	53.66±2.02$^{*, \#, @}$	0.66±0.08$^{*, \#, c}$
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	30.00±5.77$^{*, b, c}$	29.66±2.18$^{*, \#, c}$	0.40±0.10$^{*, b, c}$
Group H (120mg/kg of AMH + 500 mg/kg of MET)	28.33±1.66$^{*, \#,}$	25.33±2.02$^{*, \#}$	0.50±0.05$^{*, b}$
F-Ratio	14.88	79.11	14.54

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*.

SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihotesculenta*, *: significant when compared with group A(control group), ^{a}: not not-significant when compared with group A(control group), $^{\#}$: significant when compared with group D(diabetic group), ^{b}: not significant when compared with group D(diabetic group), $^{@}$: significant when compared with group H(metformin group), ^{c}: not significant when compared with group D(metformin group).

The mean FSH, LH and Testosterone level of the study are shown in table 4.6

FSH level

The analysis indicated that group B, C, D, G and H (p=0.00, p=0.00, p=0.00, p=0.00) had a low statistically significant difference when compared with group A(control) while groups E and F had no statistically significant difference when compared to group A(control). However, groups A, E, and F (p=0.01, p=0.00, p=0.01) had a high statistically significant

difference and group H (p=0.04) had a low statistically significant when compared with group D(diabetic) while groups B, C and G had no statistically significant difference when compared with group D(diabetic). Furthermore, the data also indicated that groups A, D, E, and F (p=0.00, p=0.04, p=0.00, p=0.00) had a high statistically significant difference when compared with group H(metformin) in contrast, groups B,C and G had no statistically significant difference when compared with group H (metformin).

LH

The analysis indicated that group B, C, D, E, F, G and H (p=0.00, p=0.00, p=0.00, p=0.00, p=0.00, p=0.00, p=0.00) had a low statistically significant difference when compared with group A(control). However, groups A, E, and F (p=0.00, p=0.03, p=0.00) had a high statistically significant difference and groups B, C, G and H (p=0.00, p=0.00, p=0.00, p=0.00) had a low statistically significant when compared with group D(diabetic). Furthermore, the data also indicated that groups A, D, E, and F (p=0.00, p=0.04, p=0.00, p=0.00) had a high statistically significant difference and group B(p=0.03) had a low statistically significant difference when compared with group H(metformin) in contrast, groups C and G had no statistically significant difference when compared with group H(metformin).

Testosterone

The analysis indicated that group B, C, D, E, F, G and H (p=0.00, p=0.00, p=0.00, p=0.00, p=0.00, p=0.00, p=0.00) had a low statistically significant difference when compared with group A (control). However, groups A, E, and F (p=0.00, p=0.03, p=0.00) had a high statistically significant differencewhen compared with group D(diabetic) while groups B, C, G, and H had no statistically significant difference when compared to group D(diabetic). Furthermore, the data also indicated that group A (p=0.00) had a high statistically significant difference when compared with group H(metformin) in contrast, groups B, C, D. E, F and G had no statistically significant difference when compared with group H (metformin).

Table 4.7: effect of methanolic seed extract of *Maniholtesculenta* on Active motile, Non-motile and Total sperm count in alloxan diabetic rats.

	Active motile(%)	Non-motile(%)	Total sperm count (x10^6/ml
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	96.33±0.66$^{\#, c}$	3.66±0.66$^{\#, @}$	73.26±3.82$^{\#, @}$
Group B (100 mg/kg of MSME)	95.00±1.15$^{*, \#, @}$	5.00±1.15$^{a, \#, @}$	75.56±6.89$^{a, \#, @}$

Group C (1000 mg/kg of MSME)	92.33±1.45$^{a, \#, @}$	7.66±1.14$^{*, b, c}$	77.50±1.25$^{*, b, c}$
Group D (120mg/kg of AMH)	72.33±1.45*, c	24.33±4.70$^{*, c}$	34.00±6.15$^{*, c}$
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	93.00±2.08$^{a, b, c}$	7.00±2.08$^{*, b, c}$	57.46±3.29$^{*, b, c}$
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	94.00±2.08$^{*, b, c}$	6.00±2.08$^{*, b, c}$	64.76±2.17$^{*, b, c}$
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	83.33±6.00$^{a, b, c}$	16.66±6.00$^{*, b, c}$	53.20±2.91$^{*, b, c}$
Group H (120mg/kg of AMH + 500 mg/kg of MET)	90.66±5.36$^{a, b, c}$	9.33±5.36$^{*, b}$	60.16±6.12$^{*, b}$
F-Ratio	6.48	3.97	10.11

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*.

SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihotesculenta*, *: significant when compared with group A(control group), ^{a}: not not-significant when compared with group A(control group), $^{\#}$: significant when compared with group D(diabetic group), ^{b}: not significant when compared with group D(diabetic group), $^{@}$: significant when compared with group H(metformin group), ^{c}: not significant when compared with group D(metformin group).

The mean Active motile, Non-motile and Total sperm count of the study are shown in table 4.7.

Active motile

The analysis indicated that groups D and G (p=0.00, p=0.01) had a low statistically significant difference when compared with group A(control) while groups B, C, E, F and H had no statistically significant difference when compared to group A(control). However, groups A, B, C, E, F, G and H (p=0.00, p=0.00, p=0.00, p=0.00, p=0.00 p=0.02, p=0.00) had a high statistically significant differencewhen compared with group D (diabetic). Furthermore, the data also indicated that groups D (p=0.00) had a low statistically significant difference when compared with group H (metformin) in contrast, groups A, B,C, E, F and G had no statistically significant difference when compared with group H (metformin).

Non-motile

The analysis indicated that groups D and G (p=0.00, p=0.01) had a high statistically significant difference when compared with group A(control) while groups B, C, E, F and H had no statistically significant difference when compared to group A(control). However, groups A, B, C, E, F, and H (p=0.00, p=0.00, p=0.00, p=0.00, p=0.00, p=0.00) had a low statistically significant differencewhen compared with group D (diabetic) while group G had no statistically significant difference. Furthermore, the data also indicated that groups D (p=0.00) had a high statistically significant difference when compared with group H (metformin) in contrast, groups A, B, C, E, F and G had no statistically significant difference when compared with group H (metformin).

Total sperm count

The analysis indicated that groups D, E and G (p=0.00, p=0.02, p=0.02) had a low statistically significant difference when compared with group A(control) while groups B, C, F and H had no statistically significant difference when compared to group A(control). However, groups A, B, C, E, F, G and H (p=0.00, p=0.00, p=0.00, p=0.02, p=0.00 p=0.00, p=0.00) had a high statistically significant differencewhen compared with group D (diabetic). Furthermore, the data also indicated that groups B, C and D (p=0.02, p=0.01, p=0.00) had a low statistically significant difference when compared with group H (metformin) in contrast, groups A, E, F and G had no statistically significant difference when compared with group H (metformin).

Table 4.8: effect of methanolic seed extract of *Maniholtesculenta* on normal Sperm cells and Abnormal sperm cells in alloxan diabetic rats.

	Normal sperm cells (%)	Abnormal sperm cells (%)
	MEAN±SEM	MEAN±SEM
Group A (control)	95.00±1.15$^{\#,c}$	5.00 ±1.15$^{\#,c}$
Group B (100 mg/kg of MSME)	92.66±1.45$^{a,\#,c}$	7.33±1.45$^{a,\#,c}$
Group C (1000 mg/kg of MSME)	95.66±0.66$^{a,\#,c}$	4.33±0.66$^{a,\#,c}$
Group D (120mg/kg of AMH)	70.00±2.88$^{*,@}$	26.66±6.00$^{*,@}$
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	86.66±4.40$^{a,\#,c}$	13.33±4.40$^{a,\#,c}$
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	94.00±1.00$^{a,\#,c}$	6.00±1.00$^{a,\#,c}$
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	84.00±7.81$^{a,\#,c}$	16.00±7.81^{a,b,c}
Group H (120mg/kg of AMH + 500 mg/kg of MET)	91.00±5.50$^{a,\#,c}$	9.00±5.50$^{a,\#}$
F-Ratio	4.73	3.00

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*.

SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihotesculenta*, [*]: significant when compared with group A(control group), [a]: not significant when compared with group A(control group), [#]: significant when compared with group D(diabetic group), [b]: not significant when compared with group D(diabetic group), [@]: significant when compared with group H(metformin group), [c]: not significant when compared with group D(metformin group).

The mean Normal sperm cells and abnormal sperm cells of the study group is shown in table 4.8.

Normal sperm cells

The analysis indicated that group D (p=0.00, p=0.01) had a low statistically significant difference when compared with group A (control) while groups B, C, E, F, G and H had no statistically significant difference when compared to group A(control). However, groups A, B, C, E, F, G and H (p=0.00, p=0.00, p=0.00, p=0.00, p=0.00 p=0.02, p=0.00) had a high statistically significant differencewhen compared with group D (diabetic). Furthermore, the data also indicated that groups D (p=0.00) had a low statistically significant difference when compared with group H(metformin) in contrast, groups A, B, C, E, F and G had no statistically significant difference when compared with group H (metformin).

Abnormal sperm cells

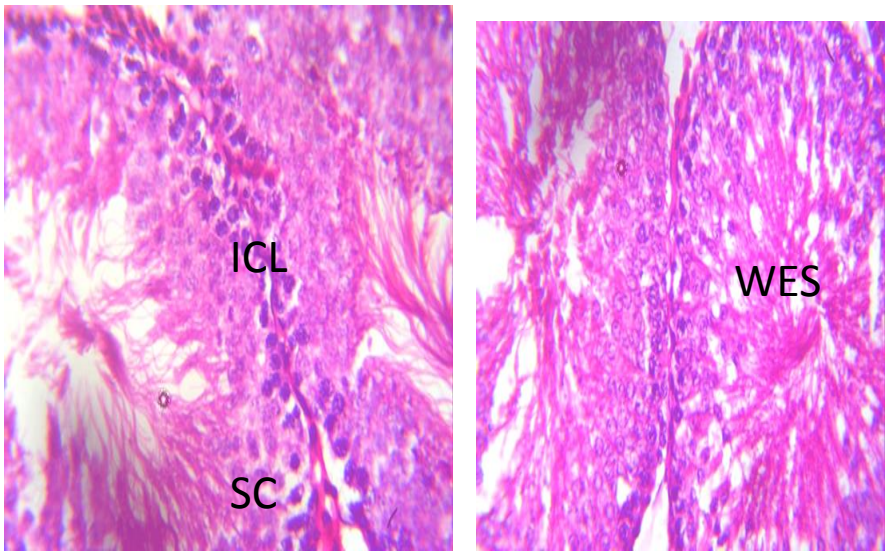
The analysis indicated that groups D (p=0.00) had a high statistically significant difference when compared with group A(control) while groups B, C, E, F. G and H had no statistically significant difference when compared to group A (control). However, groups A, B, C, E, F, and H (p=0.00, p=0.00, p=0.00, p=0.04, p=0.00, p=0.01) had a low statistically significant differencewhen compared with group D (diabetic) while group G had no statistically significant difference. Furthermore, the data also indicated that groups D (p=0.01) had a high statistically significant difference when compared with group H (metformin) in contrast, groups A, B, C, E, F and G had no statistically significant difference when compared with group H (metformin).

Table 3.1: Acute toxicity of methanolic seed extracts of *Manihot esculenta.*

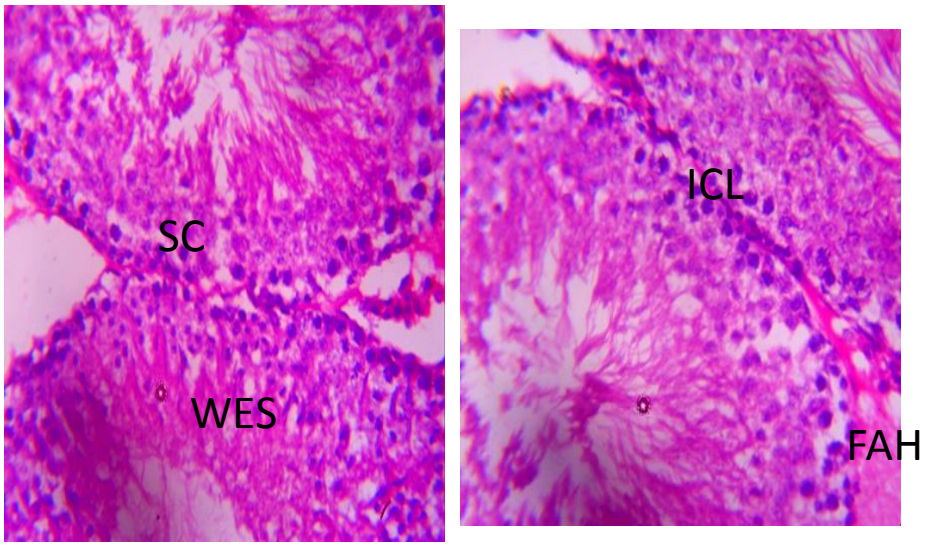
PHASE	DOSE	DEATH	OBSERVATION
1	10mg/kg	0/3	The rats remained normal
	100mg/kg	0/3	The rats remained normal
	1000mg/kg	0/3	The rats remained normal
2	1200mg/kg	0/1	The rats remained normal
	1600mg/kg	0/1	The rats remained normal
	2900mg/kg	0/1	The rats remained normal
	5000mg/kg	0/1	No death occurred

Table 3.1 result revealed that the acute toxicity (LD_{50}) of methanolic seed extracts of *Manihotesculenta* via oral route is above 5000mg/kg.

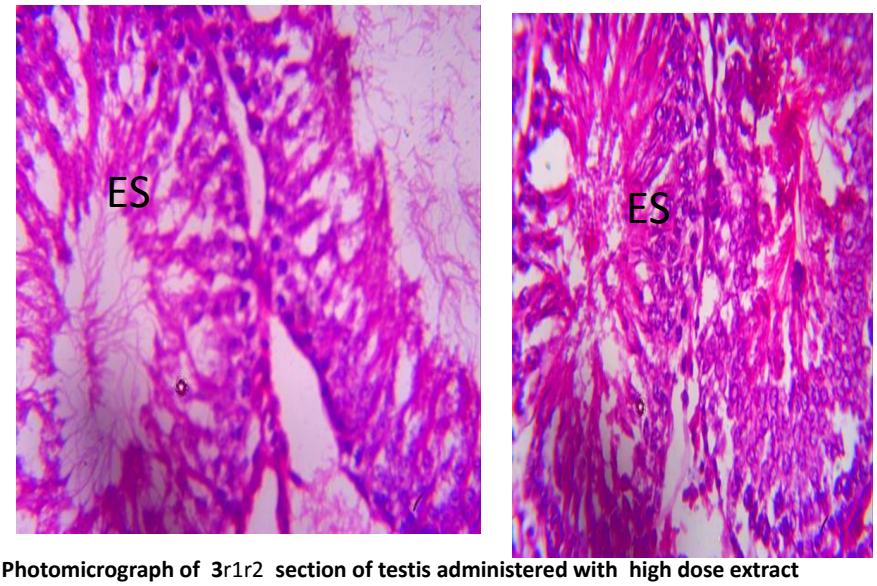
HISTOPATHOLOGICAL FINDINGS



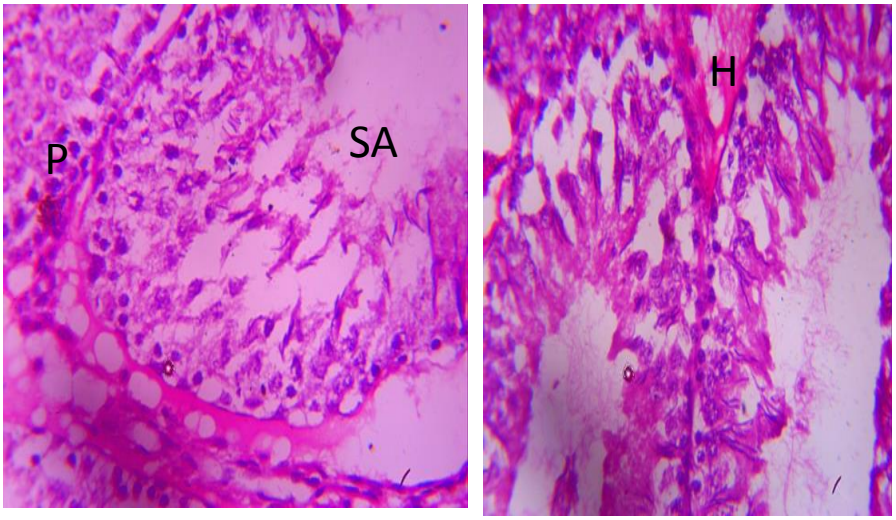
Photomicrograph of Ar1r2 control section of testis (x400)(H/E) shows normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES).



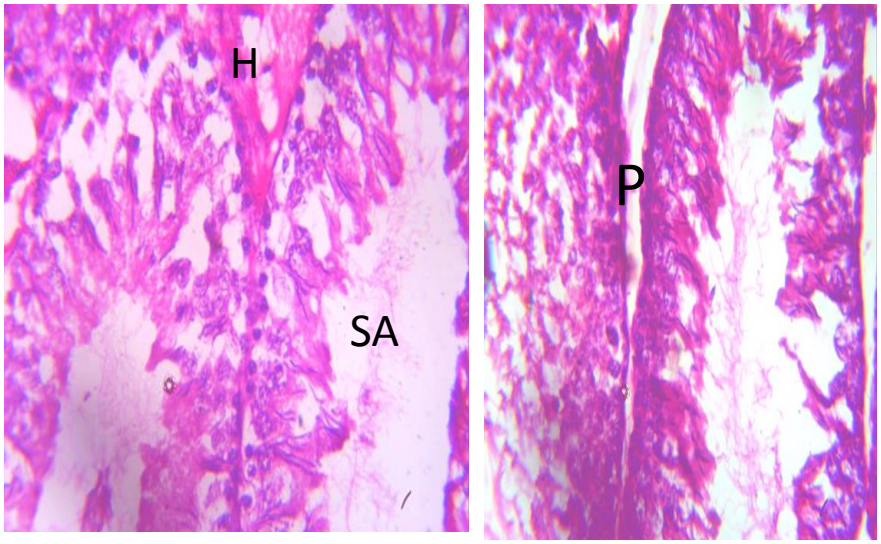
Photomicrograph of Br1r2 section of testis administered with low dose extract (x400)(H/E) shows mild focal area of hemorrhage (FAH) otherwise normal with active seminiferous tubules that are lined with interstitial cells of the leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES).



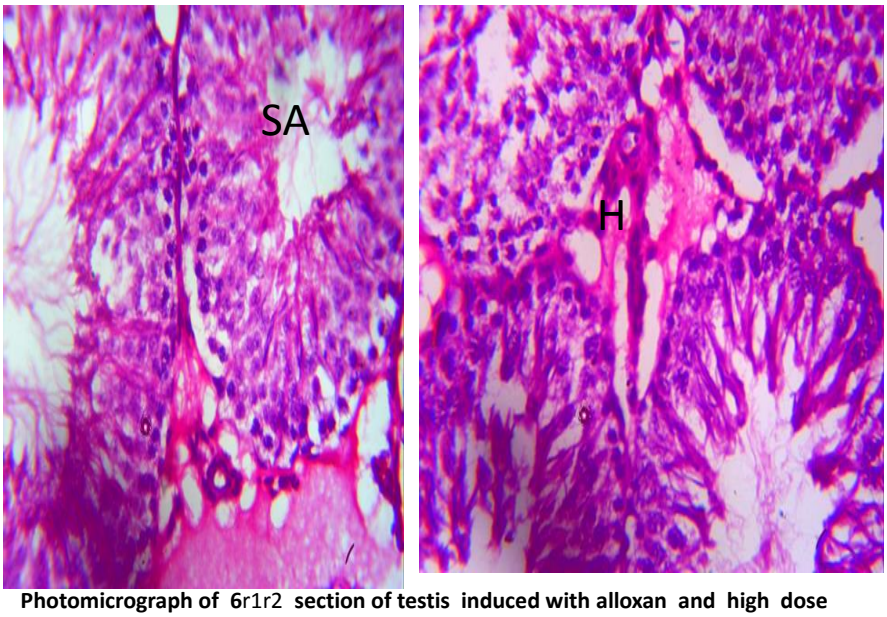
Photomicrograph of 3r1r2 section of testis administered with high dose extract (x400)(H/E) shows testicular tissue with active seminiferous tubules that are lined with active testicular cells and moderately enhanced spermatogenesis (ES).



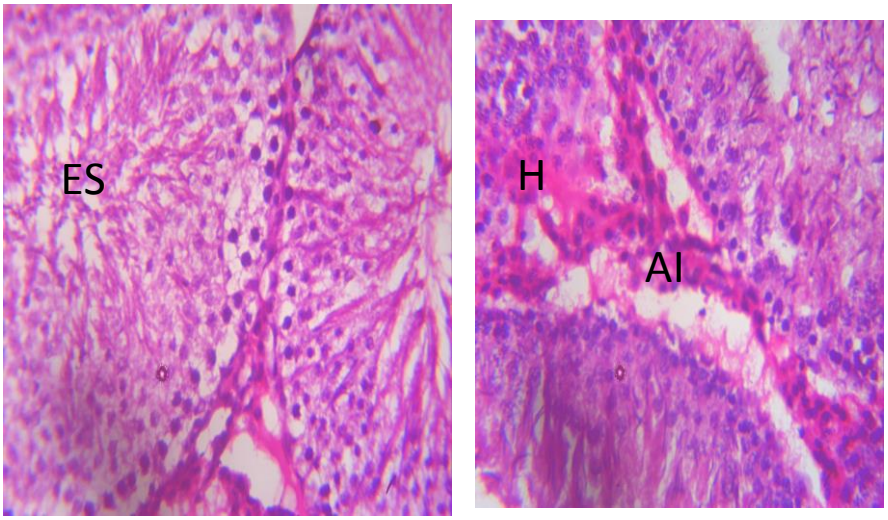
Photomicrograph of 4r1r2 section of testis induced with alloxan (x400)(H/E) shows testicular tissue with severe spermatogenic arrest (SA) inactive seminiferous tubules , severe pykotic (P) testicular cell and moderate focal area of hemorrhage (H).



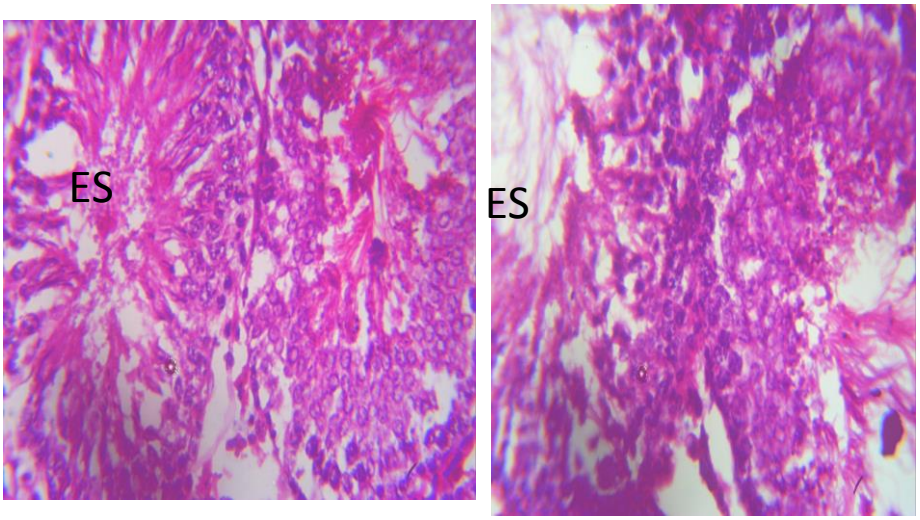
Photomicrograph of 5r1r2 section of testis induced with alloxan and low dose (x400)(H/E) shows mild regeneration with testicular tissue with moderate spermatogenic arrest (SA) , moderate pykotic (P) testicular cell and moderate focal area of hemorrhage (H).



Photomicrograph of 6r1r2 section of testis induced with alloxan and high dose (x400)(H/E) shows moderate regeneration with active testicular cell with mild spermatogenic arrest (SA) and moderate focal area of hemorrhage (H).



Photomicrograph of 7r1r2 section of testis induced with administered with high dose extract then alloxan (x400)(H/E) shows moderate protection with active testicular cell, moderately enhanced spermatogensis (ES) and moderate focal area of hemorrhage (H) with aggregate of inflammation (AI)



Photomicrograph of 8r1r2 section of testis induced with alloxan and standard drug (x400)(H/E) shows moderate regeneration with active testicular cell and moderately enhanced spermatogensis (ES).

4.3 Discussion of the Findings

In this study, we discovered a reduction in the body weight as shown by the group D, this implying that alloxan monohydrate impacts on the weight of the organ thereby causing its reduction. This reduction in weight of alloxan induced diabetic rats agrees with the research conducted by Min Jeong Kim and Bae Jin Ha (2013). *Manihot esculenta* leaves a positive impact on the weight of the animals when initial weight was compared to final weight. Group E which was induced with Alloxan monohydrate and then treated with *Manihot esculenta* showed a non significant increase in weight. This might imply that *Manihot esculenta* has a role to play in the recovery of the weight lost as a result of alloxan monohydrate. 1000mg/kg of the extract when taken before inducing alloxan shows a highly significant increase in weight, signifying that the extract possesses ameliorative properties. The could be linked to the phytochemical components of the *Manihot esculenta*. We also observed a significant increase in weight of diabetic animals treated with metformin. According to the studies conducted by Danielle *et al*, metformin does not directly impact on weight loss or weight gain, since it is usually administered after the administration of alloxan, it is assumed that the weight loss occurs as a result of the diabetes that has been induced and the significant increase in weight noticed shows an improvement and its effectiveness in balancing off the effect of alloxan monohydrate. Akhtar Ali *et al* reported there was no significant difference in weight loss following the administration of metformin to diabetic groups.

For the relative weight of testes, when compared with the control group (Group A), there was no significant difference in organ weight of other groups, this signifies that alloxan, methanolic extract of *Manihot esculenta* and metformin does not impact any significant effect on the weight of the testes of the animal.

In the hormonal assay, we discovered that the methanolic seed extract of *Manihot esculenta* played a major role in restoring the FSH level after induction of Alloxan monohydrate and treatment with *Manihot esculenta* seed extract as seen when Group E (Alloxan + Low extract dose) and F (Alloxan + High extract dose) is compared with the control group (Group A). Alloxan monohydrate reduces FSH level (citation). Treating with metformin reduces FSH level. Inducing with only extract reduces FSH level as seen in Group B and C.

We also discovered a decrease in leutinizing hormone across all the groups. However Group A, E and F has a high significant difference when compared with group D, this signifies that there was an increase in the level of leutinizing hormone following the treatment of Alloxan monohydrate. Inducing with alloxan monohydrate reduces the hormonal level whereas treatment with low dose and high dose of methanolic seed extract of *Manihot esculenta* restores the hormonal level. We also discovered that inducing this extract without having a significant condition, in this case, diabetes might cause hormonal imbalance or reduction as the case maybe.

Testosterone when compared to other groups, showed a significant reduction across the groups. This could be as a result of physiological changes that occur in the body of the animal as a response to induction. When compared to the diabetic group, Group E(Alloxan + low dose) and F(Alloxan +High dose) showed a significant difference.

Sperm Motility

The diabetic group (Group A) and Group G, shows a significantly low motility when compared to the control. This suggests that the alloxan-induced diabetics affect sperm motility this agrees with Sexton and Jarow (1997). It also suggests that the methanolic seed extract of *Manihot esculenta* does not have a protective function to this effect, as conveyed by its impact on Group G. We observed the highest number of active sperm in the control group A, whereas we observed the higher number of inactive sperm in the diabetic group D. We observed the lowest number of active sperm in diabetic group D and observed the lowest number of inactive sperm in control group A. This agrees with the literatures that states that

diabetes affects sperm activity. When compared to the control group, Group B, C, E and F showed a high level of motility. This implies that methanolic seed extract of *Manihot esculenta* increases sperm motility and reverses the effect alloxan had on the sperm motility. Group G showed an increase in inactive sperm. This could be as a result of the alloxan induced after the induction of the seed extract. This goes to suggest that the extract does not have a protective function against the effect of alloxan on sperm activity.

Sperm count

Findings from the study show that Alloxan induction results to low sperm count, this is supported by the findings of Sexton and Jarow (1997).

Sperm count in the groups treated with Low dose MSME, High dose of MSME and Metformin shows an improvement in the sperm count of the animal. We also noticed a high sperm count in the groups that received the extract alone. This suggest that the extract may possess properties that improves sperm count.

Sperm morphology

The control group has the highest percentage of normal sperm cell and the lowest percentage of abnormal sperm cell. The group induced with alloxan shows the lowest percentage of normal sperm cell and the highest percentage of abnormal sperm cell. The groups that were given methanolic seed extract of *Manihot esculenta* showed a high percentage of normal sperm cells and very low percentage of abnormal cells. This signifies that the seed extract does not hinder fertility. Whereas, the induction of alloxan monohydrate reduces male fertility.

In this study also, findings were made as it regards the acute toxicity of methanolic seed extract of *Manihot esculenta* and result revealed that the acute toxicity (LD_{50}) of methanolic seed extracts of *Manihotesculenta* via oral route is above 5000mg/kg.

Histologically

For the testes, findings showed a normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the leydig, sertoli cells and well enhanced spermatogenesis.

When administered with low dose extract, the sample showed mild focal hemorrhage, normal with active seminiferous tubules lined with interstitial cells of leydig and sertoli and well lined spermatogenesis. The reason for the focal hemorrhage is not exactly defined.

The section of testes administered with high dose extract shows normal tissue cells and moderately enhanced spermatogenesis. The groups induced with alloxan showed testicular tissues with severe spermatogenic arrest, inactive seminiferous tubule, severe pykotic testicular cell and moderate focal area of hemorrhage. This signifying that the induction of alloxan affects the testes, suggesting that spermatogenic arrest of testicular tissues can arise as an adverse effect of inducing diabetes. This might be the reason for the reduction of sexual activity in a patient with a history of long years of diabetes, especially when not properly managed.

The sections treated with Low dose, High dose and Metformin showed regeneration of tissue, this signifying the anti-inflammatory properties of the extract and the role it can play in reversing the effect of diabetes induced by alloxan.

CHAPTER FIVE

5.1 SUMMARY, CONCLUSION, RECOMMENDATION, AND AREA FOR FURTHER STUDIES

The above study which aims at evaluating the effect of methanolic extract of *Manihot esculenta* on the testes of alloxan induced diabetic adult male wister rat, explored the effect on several parameters such as MORPHOLOGY, SEMEN ANALYSIS, HORMONAL ASSAY, AND HISTOLOGICAL ANALYSIS.

5.1.1 MORPHOLOGICAL FINDINGS

In studying the morphological parameters, it was notably discovered that the final weight of Group A, B, C, F, G and H significantly increased when compared to the initial weight. This invariably proves that he animals were not affected by the various inductions except in the case of group D which was given alloxan. This goes to show that diabetes induced by alloxan brings about a reduction in weight. This supports the findings by Ashok *et al* 2007. The weight of group E was insignificant while the weight of the extract groups proved that the weight loss by the induction of alloxan can be reversed by inducing the extracts.

Relative organ weights

The testes shows a statistical difference when compared with groups A (control), D (Alloxan) and H (Metformin).

Alloxan, methanolic extract of *Manihot esculenta* and metformin does not impact any significant effect on the weight of the testes of the animal.

5.1.2 HORMONAL ASSAY

According to the study and from the hormonal assay, we discovered that the extract, *Manihot esculenta* improves the hormonal level. This was significantly noticed in the level of FSH and LH as compared with the control group.

The methanolic seed extract of *Manihot esculenta* played a major role in restoring the FSH level after induction of Alloxan monohydrate and treatment with *Manihot esculenta* seed extract.

Treating with metformin reduces FSH level. Inducing with only extract reduces FSH level.

Inducing this extract without having a significant condition, in this case, diabetes might cause hormonal imbalance or reduction as the case maybe.

We also found out that alloxan causes an alteration in hormonal level when compared with the control group.

5.1.3 SPERM COUNT AND SPERM MOTILITY

In the group induced with alloxan, we noticed a significant alteration in the sperm count and sperm moltility when compared with the control group.

The extract proved to improve sperm count and sperm motility significantly.

5.2 CONCLUSION

Alloxan monohydrate in experimental rat resulted in male infertility characterized by altered hormonal level and decreased semen characteristics while the extract and drugs (metformin) improves this, hence, it can be used to manage testicular irregularities linked to diabetes. Metformin shows more effectiveness when compared with the seed extract of *Manihot esculenta*.

5.3 RECOMMENDATION

Having discovered the effect of *Manihot esculenta* seed extract on testes as a result of induction of diabetes, we recommend that a study be done to discover the specific roles played by each of the constituent components of the extract in reversing the effect alloxan had on the testes.

5.4 FURTHER STUDIES

Further studies should be done to determining if the hormonal imbalance observed after induction of alloxan has any impact on organs of the higher centers like the hypothalamus and the pituitary gland.

It is also important we do a comparative study on the link between the effect of diabetes on the testes, pituitary and hypothalamus with respect to hormonal activities.

5.5 CONTRIBUTION TO KNOWLEDGE

The study revealed to us that inducing diabetes with alloxan monohydrate can cause noticeable cellular damages in several organs, in this case the testes.

We also discovered the important function of the extract on sperm motility and sperm count.
We also noticed the extract can reverse several cellular dysfunctions that results from inducing diabetes with alloxan monohydrate.

REFERENCES

1. Agulhon, C., Agulhon, C., Petravicz, J., McMullen, A. B., Sweger, E. J., Minton, S. K., Taves, S. R., Casper, K. B., Fiacco, T. A., and McCarthy, K. D. What is the role of astrocyte calcium in neurophysiology? Neuron, 2008; 59: 932–46.
2. Ali A, Shaheen S, Zahid N, Zafar U, Ahmad F, Farooq L. Effects Of Metformin On The Weight Of Healthy And Streptozotocin-Induced Diabetic Animal Model. J Ayub Med Coll Abbottabad. Oct-Dec, 2021; 33(4): 572-576. PMID: 35124910.
3. Ali ZH. Health and Knowledge Progress among Diabetic Patients after Implementation of a Nursing Care Program Based on Their Profile. J Diabet Metabol, 2011; 2: 121.
4. Danielle Diniz Vilela, Leonardo Gomes Peixoto, Renata Roland Teixeira, Nathalia Belele Baptista, Douglas Carvalho Caixeta, Adriele Vieira de Souza, Hélen Lara Machado, Mariana Nunes Pereira, Robinson Sabino-Silva, Foued Salmen Espindola, "The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats", Oxidative

Medicine and Cellular Longevity, vol. 2016, Article ID 6978625, 9 pages, 2016. https://doi.org/10.1155/2016/6978625

5. de Oliveira EJ, de Oliveira SAS, Otto C, Alicai T, de Freitas JPX, Cortes DFM, et al. A novel seed treatment-based multiplication approach for cassava planting material. PLoS ONE, 2020; 15(3).
6. Elizabeth R. Seaquist, MD. Diabetes. The Final Frontier: How Does Diabetes Affect the Brain, 2010; 59(1): 4–5.
7. Handelsman DJ, Conway AJ, Boylan LM, Yue DK, Turtle JR Testicular function and glycemic control in diabetic men. A controlled study. Andrologia, 1985; 17(5): 488-96.
8. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. Feb., 2008; 51(2): 216-26.
9. Lingli Long, Han Qiu, Bing Cai, Ningning Chen, Xiaofang Lu, Shuhui Zheng, Xiaoxin Ye, and Yubin Li. Hyperglycemia induced testicular damage in type 2 diabetes mellitus rats exhibiting microcirculation impairments associated with vascular endothelial growth factor decreased via PI3K/Akt pathway; Jan 12, 2018; 9(4): 5321–5336.
10. Maldonado KA, Alsayouri K. Physiology, Brain, 2023. [Updated 2023 Mar 17].
11. Min Jeong Kim1 and Bae Jin Hacorresponding author 2 negative multidrug-resistant bacteria. BMC Complement Altern Med., 2013; 13: 26.
12. Nita Gandhi Forouhi and Nicholas J. Wareham. Epidemiology of diabetes, 2014; 42(12): 698–702.
13. Nita Gandhi Forouhi, Nicholas J. Wareham. Epidemiology of diabetes, Medicine, 2010; 38(11): 602-606.
14. Nita Gandhi Forouhi, Nicholas J. Wareham. Epidemiology of diabetes, Medicine, 2010; 38(11): 602-606.
15. Osasenaga Macdonald Ighodaro and Abiola Mohammed Adeosun and Oluseyi Adeboye Akinloye. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies, 2017; 53(6): 365-374.
16. Osasenaga Macdonald Ighodaro, Abiola Mohammed Adeosun, Oluseyi Adeboye Akinloye. Alloxan-induced diabetes, a common model for evaluating the glycemic control potential of therapeutic compounds and plants extracts in experimental studies, Medicina, 2017; 53(6): 365-374.
17. Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee DE, Breteler MM Association of diabetes mellitus and dementia: the Rotterdam Study. Diabetologia, 1996; 39: 1392–1397.

18. Pearson, D. *Laboratory Techniques in Food Analysis* (1st edition). Butterworth, London, 1976; 40-100.
19. Pearson, D. *Laboratory Techniques in Food Analysis* (1st edition). Butterworth, London, 1976; 40-100.
20. Physical, Hematological, and Histopathological Signs of Toxicity Induced by African Medicinal Plants., in Toxicological Survey of African Medicinal Plants, 2014.
21. PMCID: PMC3834441PMID: 24278624.
22. PMCID: PMC4273195PMID: 25054973.
23. Popoola T.O.S and Yangomodou. O.D Extraction, Properties and Utilization Potentials of Cassava Seed Oil. Biotechnology, 2006; 5(l): 38-41.
24. Pouya Saeedi, Inga Petersohn, Paraskevi Salpea, Belma Malanda, Suvi Karuranga, Nigel Unwin, Stephen Colagiuri, Leonor Guariguata, Ayesha A. Motala, Katherine Ogurtsova, Jonathan E. Shaw, Dominic Bright, Rhys Williams. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. RESEARCH ARTICLE| VOLUME, 2019; 157: 107843.
25. Ram Kela, Bala Srinivasan, Melanie Davies. Glycaemic management of type 2 diabetes, 2010; 38(11): 618-625.
26. S. Lenzen The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 2008; 51(2): 216-226.
27. Sexton WJ, Jarow JP Effect of diabetes mellitus upon male reproductive function. Urology. Apr., 1997; 49(4): 508-13.
28. Tiwana MS, Leslie SW Anatomy, Abdomen and Pelvis: Testicle, 2022.