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Evaluating the Effect of Aqueous Extract of Tiger Nut,*Cyperus esculentus*(Cyperaceae)Tuberson the Adrenal Gland of Adult Male Wistar Rats.

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Abstract: *Cyperus esculentus contains phytochemicals such as alkaloids, sterols, resins, tannins etc which could serve as potential sources of vegetable drugs in herbal medicine. This study was aimed at evaluating the effect of aqueous extract of Cyperus esculentus tuberson the adrenal gland of adult male wistar rats. A total of sixteen healthy male rats were used for the experiment. They were housed in four big iron mesh cages making them four groups of four animals each of random weights and were provided with feed and water daily. Administration of extract was for a period of twenty-eight days. Group A served as the control group while group B, C and D served as the test groups. The experimental substance was administered to the rats in the test groups with the aid of cannula and syringes, orally. The extract was always administered every evening for 28 days and the animals were regularly observed. Blood from the animals were collected 24 hours after the last period of extract administration, through ocular puncture, for adrenal gland analysis. The animals were open through the abdominal region for harvesting of the adrenal glands before being put into the plain tubes containing normal saline to wash off the blood. It was then put in 10% formal saline for it to be fixed prior to histological processing. It shows a significantly lower value of the relative adrenal weight of animals in group B (0.002±0.0005) which received 1000mg/kg of aqueous extract of Cyperus esculentus, when compared to group A (0.003±0.0003) being the control group. There was also a significantly lower value of the relative adrenal weight of animals in group C (0.0017±0.0001), which received 2000mg/kg of aqueous extract of Cyperus esculentus, There was an insignificantly higher value of the relative adrenal weight in group D (0.0037±0.0025) which received 3000mg/kg of aqueous extract of Cyperus esculentus The result of serum cortisol level obtained from this study showed that there was an insignificantly higher level in group B which received 1000mg/kg of aqueous extract of Cyperus esculentus tuber when compared to group A (control group). It was also seen that there was a significantly higher serum cortisol level in group C and group D which received 2000mg/kg and 3000mg/kg of aqueous extract of Cyperus esculentus tuber respectively, when compared to group A (control group). This research has established that different doses of Cyperus esculentus tubers (tiger nut) has effect on the adrenal gland.*

Keywords: *Adrenal gland; Aqueous extract; Cyperus esculentus; Wistarrats*

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I. Introduction

1.1: BACKGROUND OF THE STUDY

Cyperus esculentus, also known as 'tiger nut' in local parlance, is a plant of the family Cyperaceae. Tiger nut is a tuber that grows freely and is consumed widely in Nigeria, other parts of West Africa, East Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (1). In Africa, tiger nut is mostly cultivated in the west, Ivory Coast, Ghana, Mali, Niger, Nigeria, Senegal and Togo where they are used primarily uncooked as a side dish (2). A widely acceptable name given to tiger nut tubers in Southern Nigeria is ''Aki Hausa" which literally describes a nut that is largely cultivated and marketed by the Hausas that dominate Northern Nigeria (3,4). The plant produces rhizomes from the base and tubers that are somewhat spherical (5). It also has many other common names such as chufa sedge, nut grass, yellow nutsedge, tigernut sedge, earth almond and Northern nutgrass (6, 7).

Tiger nut milk without sugar can be drunk for diabetes for its content in carbohydrate which is a base of sucrose and starch (without glucose) and due to its content of arginine which liberates the hormone insulin. As food, tiger nut can be eaten as snack which can be prepared by soaking in water for few minutes. It can also be eaten roasted, dried, baked and can be made into a refreshing beverage called ''Horchata De Chutas'' or tiger nut milk. It also finds uses as a flavoring agent for ice cream and biscuits (8), as well as in making oil, soap, starch and flour (9).

The tiger nut milk compared with any other soft drink is not just a refreshing drink but also very healthy. It contributes to the reduction in the cholesterol by diminishing the ‘’bad’’ cholesterol low density lipoprotein (LDL), and increasing the ‘’good’’ cholesterol, high density lipoprotein (HDL) (10). Its content of vitamin E also collaborates against the cholesterol because it has an antioxidant effect over fats, which are ideal for coronary heart disease (11). *Cyperus esculentus* was reported to help in preventing heart thrombosis and activates blood circulation, responsible for preventing and treating urinary tract and bacterial infection, assist in reducing the risk of colon cancer (9). Tiger nut milk has been found to be good for preventing arteriosclerosis, since its consumption can help prevent heart problems and thrombosis and activate blood circulation (11).

A study on the effects of ethanolic extract of *cyperus esculentus* on some liver function parameters of albino wistar rats showed that the extract could be unhealthy to the liver (12). A study on the influence of *cyperus esculentus* tubers on male rat copulatory behavior showed that it stimulated sexual motivation in both highly and moderately active rats, it improved sexual performance and increased serum testosterone level significantly after administration (13). A study on the effect of *cyperus esculentus* oil on liver, kidney and hematological biomarkers in low dose streptozocin and high fat diet exposed male wistar rats showed that *cyperus esculentus* oil exhibited hepatoprotective ability, enhances renal function and maintains hematological status (14). A study on the effect of aqueous extract of tiger nut on sperm parameters and testosterone level of male albino rats shows that aqueous extract of tiger nut has the capability of increasing the weights of the testes and epididymis, sperm count, sperm quality and testosterone level. Hence, aqueous extract of tiger nut can be used as a possible fertility booster and to attenuate sperm toxicity (15).

However, information on the effect of aqueous extract of tiger nut on the adrenal glands of albino rats was scarcely available. An assessment of the effect of *cyperus esculentus* tubers on various human glands is essential to ascertain merits and detect demerits if any in order to avert health conditions that may arise overtime due to continual use. A gland of particular interest is adrenal gland.

This research was conducted to ascertain the effect of the use of tiger nut on the histology and hormones of the adrenal glands.

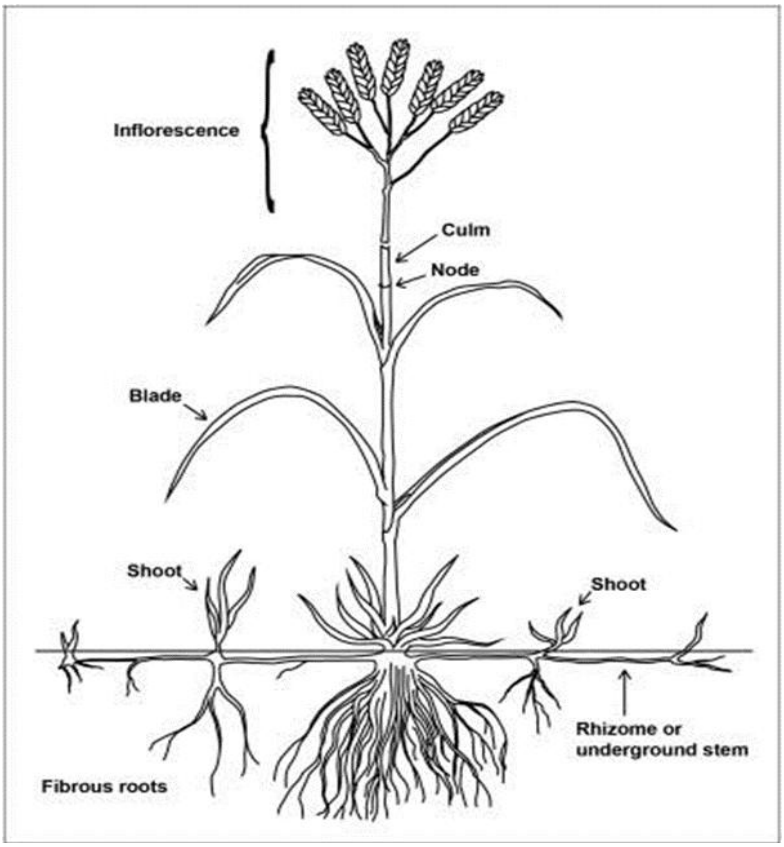
1.2: STATEMENT OF PROBLEM

The adrenal glands, located on top of the kidneys, make hormones that are essential for body functions. The outer layer (cortex) of the adrenal glands makes three types of steroid hormones. And the inner layer (medulla) of the adrenal glands produce epinephrine and norepinephrine (16). The adrenal glands release hormones that regulate metabolism, immune system function, and the salt-water balance in the blood stream; they also aid in the body’s response to stress (17).

Damage to the adrenal glands leads to disorders such as Addison’s disease, Cushing’s syndrome, pheochromocytoma and primary aldosterone (18,19).

Cyperus esculentus is a tuber that grows freely and is consumed widely in Nigeria (1). *Cyperus esculentus* contains phytochemicals such as alkaloids, sterols, resins, tannins etc. The phytochemicals of this tuber possess some biological active compounds which could serve as potential sources of vegetable drugs in herbal medicine (20).

***Cyperus esculentus* plant (Adyizi, 21).**



Labelled diagram of *Cyperus esculentus* (Kathyln, 22).

Brown variety tiger nut tubers

Small size yellow variety tiger nut tubers

Big size yellow variety tiger nut tubers

. Black variety tiger nut tubers

II. Materials and methods

2.1: location and duration of study

This study was carried out in the animal house of College of Health Science, Nnamdi Azikiwe University, Nnewi campus, Anambra State. The experimental animals were made to acclimatize for a period of fourteen days after which the experimental substance was administered for 28 days.

2.2: ethical consideration and approval

Ethical approval was obtained from the ethical committee of the faculty of basic medical sciences, Nnamdi Azikiwe University, Nnewi Campus. The experimental protocols were carefully reviewed by the supervisor of this experimental research.

2.3: Experimental animals and design

A total of sixteen healthy male rats were used for the experiment. The rats were purchased from Dr. Asomugha's animal farm in Otolo Nnewi and then transferred to the animal house of the college of health sciences, Nnamdi Azikiwe University, Nnewi. They were housed in four big iron mesh cages making them four groups of four animals each of random weights and were provided with feed and water daily. Prior to the commencement of extract administration, the animals were acclimatized for a period of 14 days and administration of extract was for a period of twenty-eight days. Group A served as the control group while group B, C and D served as the test groups.

2.4: Collection and identification of plant materials

Tiger nut tubers used for this study were purchased from Nkwo market, Otolo Nnewi in the month of August, 2019. It was identified and authenticated at the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka.

2.5: Preparation of tiger nut extract

Tiger nut tubers were obtained from Nkwo market, Otolo Nnewi. They were then washed and oven dried at 37^{o}c for 24hrs and thereafter, pulverized into smooth powder using an impact mill. The pulverized nut was mixed with relative quantity of distilled water and left for 24hrs. The mixture was stirred at 3hrs interval using a sterile rod and passed through sieving cloth. The filtrate was then concentrated to dryness under reduced temperature and pressure using a Buchner funnel and Whatman No.1 filter paper. The dried extract was stored in an air-tight container and kept in a refrigerator at 4^{o}c prior to use.

2.6: Exposure of animals to experimental substance

The animals before commencement of extract administration were properly weighed using an electronic kitchen scale (SF-400) which has a capacity of 7000g and then the extract administration commenced.

The animals were then given the extract through the following protocols:

Group A served as the control group and was fed with water and feed only

Group B received 1000mg/kg body weight, plus water and feed

Group C received 2000mg/kg body weight, plus water and feed

Group D received 3000mg/kg body weight, plus water and feed

The extract was prepared into solution, in known quantity of water before administration. The experimental substance was administered to the rats in the test groups with the aid of cannula and syringes, orally. The extract was always administered every evening for 28 days and the animals were regularly observed.

2.7: Collection of blood and extraction of gland

Blood from the animals were collected 24 hours after the last period of extract administration, through ocular puncture, for adrenal gland analysis. Their blood samples were put in plain sterile tubes. 16 animals were sacrificed by chloroform deactivation 24 hours after the last administration of the extract. The animals were open through the abdominal region for harvesting of the adrenal glands before being put into the plain tubes containing normal saline to wash off the blood. It was then put in 10% formal saline for it to be fixed prior to histological processing.

2.8: Tissue processing

After weighing the adrenal glands, they were immediately fixed in 10% formal saline in order to preserve the constituents of the cells in their normal micro- anatomical positions as well as prevent the putrefaction and autolysis of cellular constituents. The tissues were then passed through histological techniques with the hematoxylin and eosin stained sections viewed with the aid of a light microscope and then photomicrographs were produced with the aid of a pictogram. The tissues passed through the following histological techniques:

2.8.1 Fixation:
The tissues were immediately fixed with 10% formalin in order to maintain clear and consistent tissue morphological features and to enhance subsequent staining procedures.

2.8.2 Dehydration:
Dehydration is the process of removing excess water and unbound fixative from a tissue.
This process was done by passing the tissues through ascending grades of alcohol from 50%, 70%, 90% and 100% for 1-2 hours each. Unlike the lower grades of alcohol, the tissues are put in two changes of absolute (100%) alcohol. Dehydration is done in an ascending order of grades of alcohol to prevent distortion of tissue components to immediate extraction of water from the tissues.

2.8.3 Clearing:
This is the displacement of the dehydrating solution used prior. This process enables the tissue components receptive to the subsequent infiltrating medium.
The clearing agent used was xylene for a period of 1-2 hours. Xylene is miscible with alcohol and also miscible with paraffin wax, hence it is an appropriate clearing agent for this procedure.

2.8.4 Impregnation:
This stage is also called infiltration and it involves the process of replacing a clearing agent with molten paraffin wax.
The tissues were placed in a molten paraffin wax at constant temperature of 56^{o}c in an oven and were passed through two changes of paraffin wax in the oven, 4 hours for each time.

2.8.5 Embedding:
It is the process of creating tissue blocks by using an external support medium to enable microtomy. The embedding medium should be completely compatible with the in filtrating medium in order to prevent tissue section separation and to facilitate sectioning.
Metal mould was sprayed with mould release fluid. The mould was then filled with molten paraffin wax, and the tissue oriented in it immediately with warm forceps. After cooling, the tissue block was immersed into a bowl of water. It then solidifies and is removed ready for sectioning.

2.8.6 Sectioning:
This is a process of cutting thin sections from tissues with a precision machine called microtome. Thin sections are necessary because before sections can be examined under the microscope, light has to pass through them.
The tissue block was fixed in the block holder of the microtome. It was first trimmed to expose the tissue surface. Once the tissue surface was revealed, the thickness gauge was reset to 5 micrometer and the cutting of tissue ribbons began. The ribbon sections were cut and were picked up gently with a pair of forceps, put on a slide and then put in the water bath.

2.8.7 Mounting of sections on slides:
A glass slide was dipped into the water and taken beneath the tissue ribbon to pick tissue ribbon from the water bath. The water bath contained distilled water with temperature between 50-55^{o}c (note; the temperature of the water bath has to be about 5^{o}c below the melting point of the parrafin wax used in embedding the tissue). Care must be taken to ensure the tissue ribbon is well oriented at the center of the slide. 20% alcohol was then flooded on the surface of the slide on which the ribbon was. Water was drained off and the slide was put in a rack and transferred to an incubator at 37^{o}c overnight so that the section was completely fixed on the slide and become dry.

2.8.8 Staining:
This is the use of dyes to give contrasting colors to different elements of the cells or tissues. Most common dyes used are eosin and hematoxylin. Hematoxylin stains the nuclei violet while eosin stains cytoplasm pink.
Hematoxylin and eosin are used in this research. Before the sections were stained, the paraffin wax was removed so that stains will penetrate the tissues because the stains used are in aqueous solution. This is done by placing the slide in xylene which was in turn removed using alcohol because xylene is not miscible with water.

2.8.9 Mounting of cover slips on slides:
Two drops of DPX mountant was placed on an appropriate sized cover slip. The section slide which was just removed from xylene was then inverted and placed on the cover slip and then re-inverted. Trapped bubbles was avoided. The mounted cover slip slide which was air dried was then viewed under the light microscope.

2.9: Statistical analysis
The results of the data were statistically analyzed using (SPSS) windows version 21.0 software. Results are presented as mean and standard error of mean (SEM). Significant differences of result were established by one-way ANOVA. P values ($p<0.05$) at 95% confidence interval were considered as significant.

III. Results

3.1: PHYSICAL AND BEHAVIOURAL OBSERVATIONS

- At the beginning of the experiment, all the animals were apparently healthy.
- At the fifth day of acclimatization, they began to consume more feed and water. Their stools were regular, dark brown in colour, and had a firm texture.
- From the third day of administration, the rats in group B, C and D had a change in the texture and colour of their stool. The texture of their stool became soft and the colour became a little lighter. This was only noticed in the test groups
- After one week of administration of tiger nut, the rats in the test groups were seen to be active, their furs were silk and looked very healthy, as compared to the control group. There was also an increase in the weights of all the rats.
- By the third week of administration, rats in the test groups were very active and looked big and healthy.
- During the experiment, there was a general increase in weight of all animals.

Table 3.1: Changes in the body weight of the animals

ANIMAL GROUPING		ANIMAL WEIGHT(g) MEAN±SEM	WEIGHT DIFFERENCE (g)	P-VALUE	F-VALUE
GROUP A	FIRST WEEK	115.00±2.89	40		3.86
	SECOND WEEK	160.00±8.16		0.013*	
	THIRD WEEK	157.50±10.31		0.017*	
	FOURTH WEEK	155.00±17.08		0.023*	
GROUP B	FIRST WEEK	127.50±4.79	65		26.158
	SECOND WEEK	177.50±6.29		0.000*	
	THIRD WEEK	170.00±5.77		0.000*	
	FOURTH WEEK	192.50±4.79		0.000*	
GROUP C	FIRST WEEK	142.50±2.5	70		14.07
	SECOND WEEK	187.50±4.78		0.002*	
	THIRD WEEK	197.50±6.29		0.000*	
	FOURTH WEEK	212.50±13.77		0.000*	
GROUP D	FIRST WEEK	160.00±7.07	80		14.36
	SECOND WEEK	197.50±2.50		0.001*	
	THIRD WEEK	195.00±5.0		0.000*	
	FOURTH WEEK	212.50±7.5		0.001*	

Data was analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at $P \leq 0.05$ and $P > 0.05$ means not significant.

*= P value significant ≤0.05

The table shows that the initial body weight, which is at first week, of the animals in group A (control group) is 115.00±2.89. The body weight is 160.00±8.16 at the second week, 157.50±10.31 at the third week, and the final body weight (fourth week) is 155.00±17.08. The weight difference between the initial weight (first week) and the final weight (fourth week) in group A is 40g. The p-value is 0.013 at second week, 0.017 at third week, and 0.023 at the fourth week. The f-value is 3.86. The initial body weight, which is at first week, of the animals in group B is 127.50±4.79. The body weight is 177.50±6.29 at the second week, 170.00±5.77 at the third week, and the final body weight (fourth week) is 192.50±4.79. The weight difference between the initial weight (first week) and the final weight (fourth week) in group B is 65g. The p-value is 0.000 at second week, 0.000 at third week, and 0.000 at the fourth week. The f-value is 26.158. The initial body weight, which is at first week, of the animals in group C is 142.50±2.5. The body weight is 187.50±4.78 at the second week, 197.00±6.29 at the third week, and the final body weight (fourth week) is 212.50±13.77. The weight difference

Table 3.2: Effect of *Cyperus esculentus* tubers on relative organ weight of adult male wistar rats

Animal groups	N	Relative Organ Weight(g) Mean±SEM	F-value	P-value
Group A	4	0.003±0.0003		
Group B	4	0.002±0.0005	6.928	0.028
Group C	4	0.0017±0.0001		0.015
Group D	4	0.0037±0.0025		0.363

Data was analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at P<0.05 and P>0.05 means not significant.

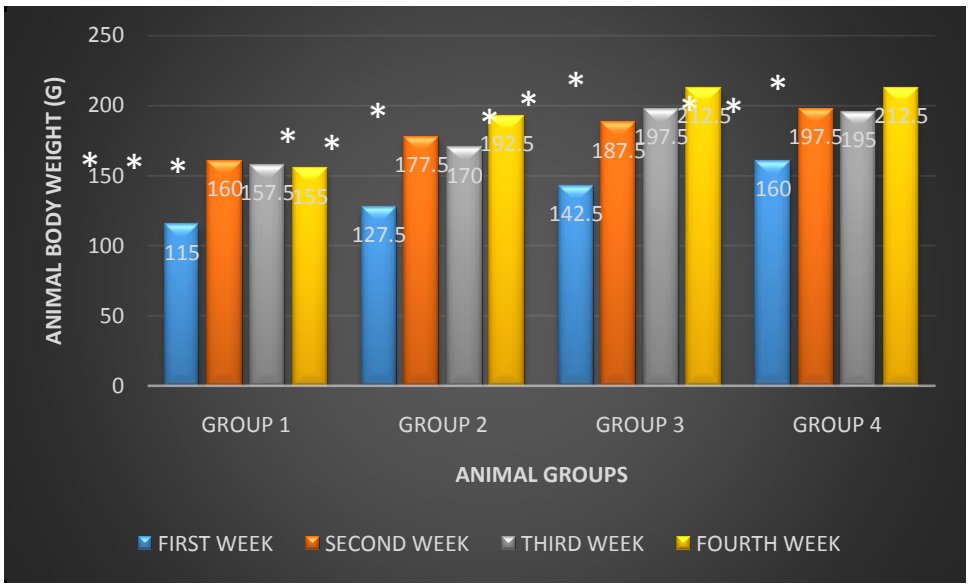
*= P value significant ≤0.05

Table 4.2 shows that the relative adrenal weight and standard error of mean of animals in group A (control group) is 0.003±0.0003. The F-Value is 6.928. The relative adrenal weight and standard error of mean of animals in group B is 0.002±0.0005, the F-value is 6.928 and the P-value is 0.028. The relative adrenal weight and standard error of mean of animals in group C is 0.0017±0.0001, the F-value is 6.928 and the P-value is 0.015. The relative organ weight and standard error of mean of animals in group D is 0.0037±0.0025, the F-value is 6.928 and the P-value is 0.363.

Result from table 4.2 shows a significantly lower value of the relative adrenal weight of animals in group B (0.002±0.0005) which received 1000mg/kg of aqueous extract of *Cyperus esculentus*, when compared to group A (0.003±0.0003) being the control group. There was also a significantly lower value of the relative adrenal weight of animals in group C (0.0017±0.0001), which received 2000mg/kg of aqueous extract of *Cyperus esculentus*, when compared to group A (0.003±0.0003). There was an insignificantly higher value of the relative adrenal weight in group D (0.0037±0.0025) which received 3000mg/kg of aqueous extract of *Cyperus esculentus* when compared to group A (0.003±0.0003).

between the initial weight (first week) and the final weight (fourth week) in group C is 70g. The p-value is 0.002 at second week, 0.000 at third week, and 0.000 at the fourth week. The f-value is 14.07. The initial body weight, which is at first week, of the animals in group D is 160.00±7.07. The body weight is 197.50±2.50 at the second week, 195.00±5.0 at the third week, and the final body weight (fourth week) is 212.50±7.5. The weight difference between the initial weight (first week) and the final weight (fourth week) in group D is 52.5g. The p-value is 0.001 at second week, 0.000 at third week, and 0.001 at the fourth week. The f-value is 14.36.

Result shows a significant increase of body weight in all groups.



*= P value significant ≤0.05.

Fig 3.1 graphical representation of the changes in body weight of animals between the first week of administration and the other test weeks.

The bar chat above shows that, at the first week of administration, the average body weight of animals in group 1 (control group), was 115g. At the second week, the average body weight of animals in group 1 increased to 160g. At the third week, the average body weight of animals in group 1 was 157g, and at the fourth week of administration, the average body weight of animals in group 1 was 155g. The average body weight of animals in group 2 at the first week of administration was 127.5g. At the second week, the average body weight of animals in group 2 increased to 177.5g. At the third week, the average body weight of animals in group 2 was 170g, and at the fourth week of administration, the average body weight of animals in group 2 was 192g. The average body weight of animals in group 3 at the first week of administration was 142.5g. At the second week, the average body weight of animals in group 3 increased to 187.5g. At the third week, the average body weight

of animals in group 3 was 197.5g, and at the fourth week of administration, the average body weight of animals in group 3 was 212.5g. The average body weight of animals in group 4 at the first week of administration, was 160g. At the second week, the average body weight of animals in group 4 increased to 197.5g. At the third week, the average body weight of animals in group 4 was 195g, and at the fourth week of administration, the average body weight of animals in group 4 was 212.5g.

Significant increase in animal body weight occurred in all the groups.

IV. Discussion

This study was carried out to investigate the effects of aqueous extract of *Cyperus esculentus* on the adrenal glands of adult male wistar rats.

During the course of extract administration, the rats in the control group (group A) showed no observable changes in their physical appearance. The animals in group B, C and D (test groups) had a change in the texture of their stool, the stools had a soft texture unlike that of group A (control group) which had a firm stool. This change in stool texture may be as a result of high insoluble fibres in tiger nut which passes through the gut without being digested and help prevent constipation and better digestion (23,24). The rats in group B, C and D (test groups) were also seen to be more active and more mobile when compared to group A (control group). The increased activity and positive mobility of the animals in the test groups could be as a result of high energy nutrients such as glucose, oleic acid, starch, fats, sugars and proteins contained in *Cyperus esculentus* (25).

From the period of acclimatization to the commencement of administration, there was significant weight gain in all groups. This is due to the fact that they were given feed with high nutritional value. Results showed significant increase in body weight of rats in group A. The increase in body weight of rats in group A (control group) which received water and animal feed only, throughout the duration of the experiment, is physiological. Results also showed a significant increase in the body weight of rats in groups B, C and D which received 1000mg/kg, 2000mg/kg and 3000mg/kg of aqueous extract of *Cyperus esculentus* respectively. The increased weight is physiologic and could also be due to increased feed and water intake observed all through the experimental period. The increase in weight of the animals suggests that there was increased accumulated calories from the normal rat diet and from the nutrient rich extracts. Although the animals used in this study were fed with normal rat diet, the aqueous extract of tiger nut might have allowed proper absorption of the nutrients which have allowed proper utilization of the nutrients. Low level of toxic principles may have stimulated appetite and increased feed utilization resulting in increased weight gain. This result is in agreement with the findings of (11), who reported that the mean weights of the animals in the test groups increased after the administration of *Cyperus esculentus.* The increase in weight of the animals is dose dependent and proportional to the dosage.In a study carried out by (11), the results showed that the increase in the body weight of wistar rats that were administered *Cyperus esculentus* tubers, was not dose dependent.

The result of relative adrenal weight obtained from this study showed that there was a significantly lower value in the relative adrenal weight of rats in group B and group C which received 1000mg/kg and 2000mg/kg of aqueous extract of *Cyperus esculentus* respectively, when compared to group A (control group) which received only water and animal feed throughout the period of the experiment. The result also showed an insignificantly higher value of the relative adrenal weight in group D which received 3000mg/kg of aqueous extract of *Cyperus esculentus* when compared to group A (control). On the other hand(26), who studied the effect of aqueous extract of tiger nut, reported a significantly higher value of relative testicular weight in the experimental group.

4.1:Conclusion

This research has established that different doses of *Cyperus esculentus* tubers (tiger nut), lead to an increase in body weight. Low dose (1000mg/kg) of the aqueous extract of *Cyperus esculentus* tubers, as observed, lead to a lesion on the histology of the adrenal gland. Aqueous extract of *Cyperus esculentus* tubers at doses of 2000mg/kg and 3000mg/kg, lead to higher cortisol levels in the blood, and has no adverse effect on the histology of the adrenal gland.

4.2: Recommendations

I .Further research be carried out on the adrenal gland using other extracts of *Cyperus esculentus* tuber to determine their effect on its histology and function.

II More research be carried out to determine the cause of lesion on the histology of the adrenal gland which was observed in group B in this research.

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