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Dystrophy and Vulva Cancer

Murray-Jackson-Lawler Syndrome

Highlights

Awareness Regarding Diabetes

Functional Dietary Supplementation

Discovering Thoughts, Inventing Future

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Search of Ways to Improve the Efficiency of the Diagnostic Results and the Quality of Treatment of Dystrophy and Vulva Cancer

By Cherenkov V. G., Sannikova M. V., Alexandrova I. V., Pacewicz K. G K. G. Pacwich & Egorova E. S.

Jaroslav-the- Wise Novgorod State University

Abstract- With vulvar dysplasia, sclerotic deprive and suspected cancer under our observation there were respectively 115 and 97 patients. The effectiveness of treatment of vulva dystrophy (VIN II-III degree) by the method of photodynamic therapy (PDT) 45.8=4.7%. However, dysplasia II - III degree, sclerotic changes with the formation of pronounced horn scales prevents the full PDF and recurrence of the disease. Therefore, the treatment of choice is surgical treatment with a reconstructive plastic. In order to reduce bleeding and antiblastic we used krioapplikatsiya, apparate "Harmonics", and the decay of tumor angiographic chemoembolization before surgery. The use of new reconstructive plastic surgery, including with the use of abdominal skin and fascial flap combined with vascularized lower segments of the rectus muscles (*patent for invention* **№** *2580665 from 11.11.14*), have helped to reduce complications, improve cosmetic effect and reduce the duration of lymphorrhea in 2-3 days.

Keywords: vulvar cancer, PDT, cryoapplication, apparate "Harmonics", angiohemoembolization, reconstructive plastic surgery of the abdominal skin and lower segments of the rectus muscles.

GJMR-F Classification: NLMC Code: WB 141



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Search of Ways to Improve the Efficiency of the Diagnostic Results and the Quality of Treatment of Dystrophy and Vulva Cancer

Cherenkov V. G. [α], Sannikova M. V. [σ], Alexandrova I. V. [ρ], Pacewicz K. G K. G. Pacwich [Ω] & Egorova E. S. [¥]

Abstract- With vulvar dysplasia, sclerotic deprive and suspected cancer under our observation there were respectively 115 and 97 patients. The effectiveness of treatment of vulva dystrophy (VIN II-III degree) by the method of photodynamic therapy (PDT) 45.8=4.7%. However, dysplasia II - III degree, sclerotic changes with the formation of pronounced horn scales prevents the full PDF and recurrence of the disease. Therefore, the treatment of choice is surgical treatment with a reconstructive plastic. In order to reduce bleeding and antiblastic we used krioapplikatsiya, apparate "Harmonics", and the decay of tumor angiographic chemoembolization before surgery. The use of new reconstructive plastic surgery, including with the use of abdominal skin and fascial flap combined with vascularized lower segments of the rectus muscles (*patent for invention № 2580665 from 11.11.14*), have helped to reduce complications, improve cosmetic effect and reduce the duration of lymphorrhea in 2-3 days.

Keywords: vulvar cancer, PDT, cryoapplication, apparate "Harmonics", angiohemoembolization, reconstructive plastic surgery of the abdominal skin and lower segments of the rectus muscles.

I. Introduction

Cancer of the external genitals is up to 8 % in the overall structure of the incidence of malignant neoplasms of female genital organs, relapses that occur within the first 5 years to 60 %. [1]. Vulva cancer (CV) is mainly detected in elderly menopausal women. Unfortunately, it is increasingly common at a younger age, associated with the increasing threat of papillomavirus infection [2]. And this is a reality that we have to reckon with. Features of development, multicentricity, and often diffusivity of the lesion against the background of diffuse dysplastic changes and sclerotic lichen or papillomatosis create certain difficulties in the early stages of diagnosis [8]. Given the above, we used the technique of scraping with a scalpel consisting of two stages: 1) removal (scraping) Horny scales of the epithelium of the 4-5 most suspicious areas; 2) scarification and obtaining cells from deep layers within the basal layer before the appearance of "dews" blood. Cytological examination of vulvar dysplasia allowed at 76.0±3.4% of cases to establish a correct diagnosis, including 13 cases against this background, the identified cancer (0-1 stages, which is significantly higher than the cumulative literature data (up to 57%) [3]. The study of methods of reconstructive plastic surgery (RPO) with a skin-fascial flap from the posterior thigh was started in the 80s years last century by Knapstein P. G [9].

The possibilities of treatment of vulvar dysplasia and cancer are contradictory.

II. Aim

Assessment and the role of the VDT and the search of ways of increase of efficiency of diagnostics and surgical procedures in complex therapy of vulvar dystrophies and vulvar cancer.

III. Material and Methods

With vulvar dysplasia and sclerotic lichen under our supervision were 115 patients aged 46 to 82 years and 9 patients in combination with carcinoma in situ and RV 1-II stages. Mean age 55±3.6%. PDT was performed in 43 patients with diffuse vulvar dysplasia, incl. (VIN I - 27, VIN II - 16) and in RV in order to prepare the surrounding tissues (in the transition of sclerotic changes) on the skin of inguinal masonry and inner thighs), incl. in 2 patients with relapse of CV.

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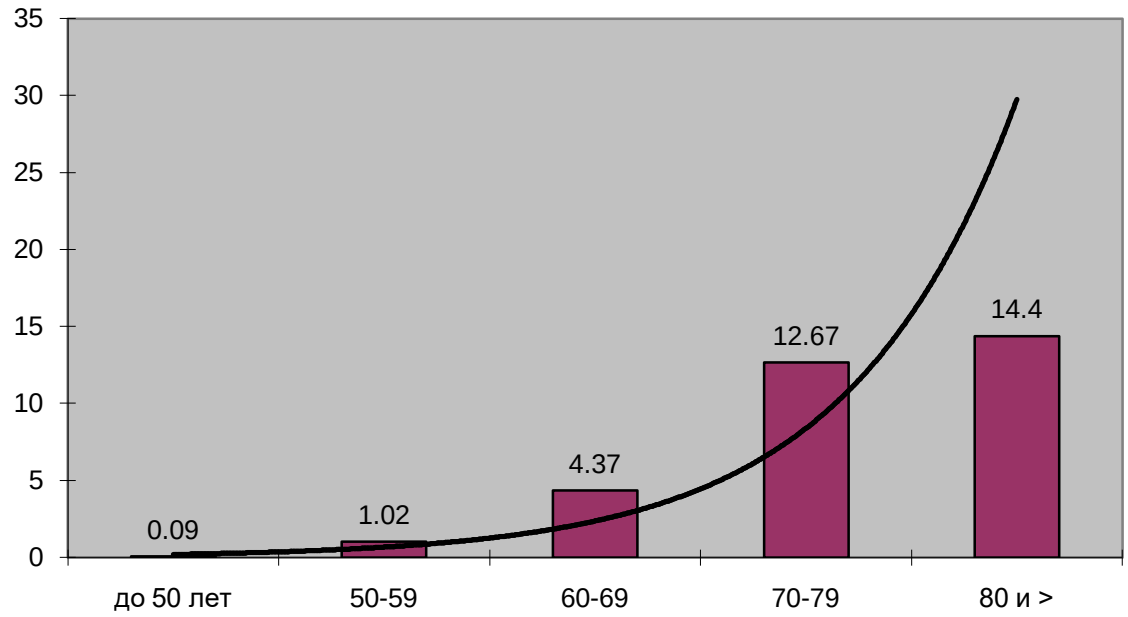


Fig. 1: The incidence of CV diseases per 100 000 of the female population of the region by age groups on average for the last 5 years (2014-2018).

In our study we used a second generation sensitizer – photoditazine. PDT session was performed 1.5-2 hours after intravenous drip infusion of the drug (VETA-Grand LLC) at the rate of an average of 1.0 mg/kg body weight in 100 ml of 0.9% sodium chloride solution in a semi-darkened room. Laser irradiation using semiconductor device "Atkus - 2" with energy density of 80-250 j/cm^2 and exposure time from 10 to 30 min. PDT allowed to cure VIN I in 21(77.7=2.2%) and only in 2 of 16 (12.5=0.5%) women with VIN II degree.

However, after 5 years, the resumption of skin itching and residual focal leukoplakia were noted in 5 women, which is associated with insufficient exposure to laser exposure to each zone, in the presence of pronounced Horny scales. In addition, PDT by moving the laser beam by hand does not avoid subjectivity, and means of disease recurrence.

5-year relapse-free survival was found in 16 of 27 (59.2±3.6%) women with predominantly VIN I. In situations with diffuse or transient II - III degree dysplasia behind the femoral fold, in our opinion, a combined approach is required in the choice of treatment depending on the prevalence of the process (PDT+ vulvectomy with RPO).



Fig. 2: Cancer in situ on the background of diffuse sclerotic lichen and vulvar dysplasia, passing to the skin of the thigh (P., 62 years).

As note themselves women after PDT was only a temporary subjective effect, after which itching resumed and they resorted to various popular their means. Plasty with full-fledged skin-fascial flaps with good vascularization from the posterior-medial surface of the thigh in 97 patients showed that it is pathogenic, since one of the main reasons for the development of VIN I-III is the sclerosis of local vessels and tissue atrophy. All patients underwent expanded vulvectomy and bilateral or unilateral inguinal-femoral lymph node dissection depending on the results of ultrasound and MRI.

Despite the obliteration of arterial vessels in the formation of tabloid dystrophy, due to anatomical features in the area of the vulva is not only preserved, but also expanded venous plexus with outflow through V. pudendi interna. In order to avoid dispersion and devitalization of the tumor cells (ablastic and antiblastic)

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excision always conducted radiowave scalpel and apparatus "Harmonics", making the preliminary incision of the skin usual scalpel.

Recently, the excision of the tumor in 37 women was preceded by cryoapplication and credibilitate to t - 185^{o} through the entire thickness of the tumor using the apparatus ERBE-6, exposure 5-10 min (Fig. 3). The data show that this approach is promising and does not affect healing. Surgical excision is almost without bloodshed on the part of the tumor. In the latter group, within 5 years, relapses occurred only in 3 patients (8.1±0.9%), which is significantly lower than in the literature.

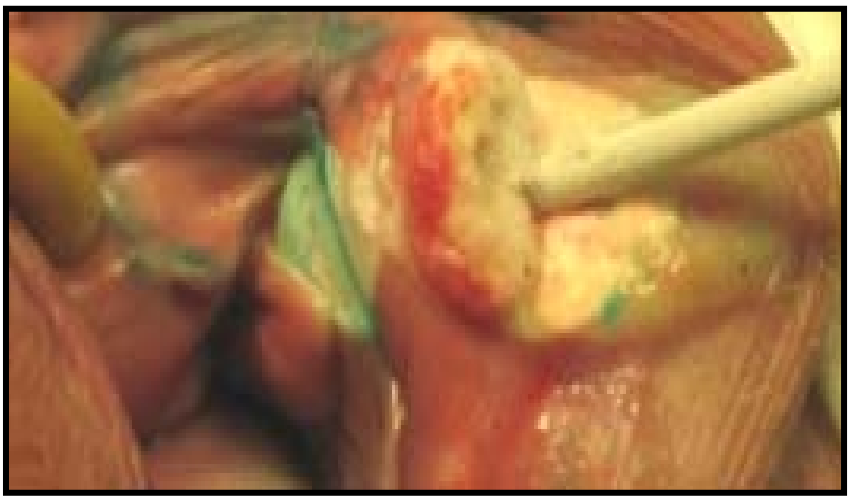
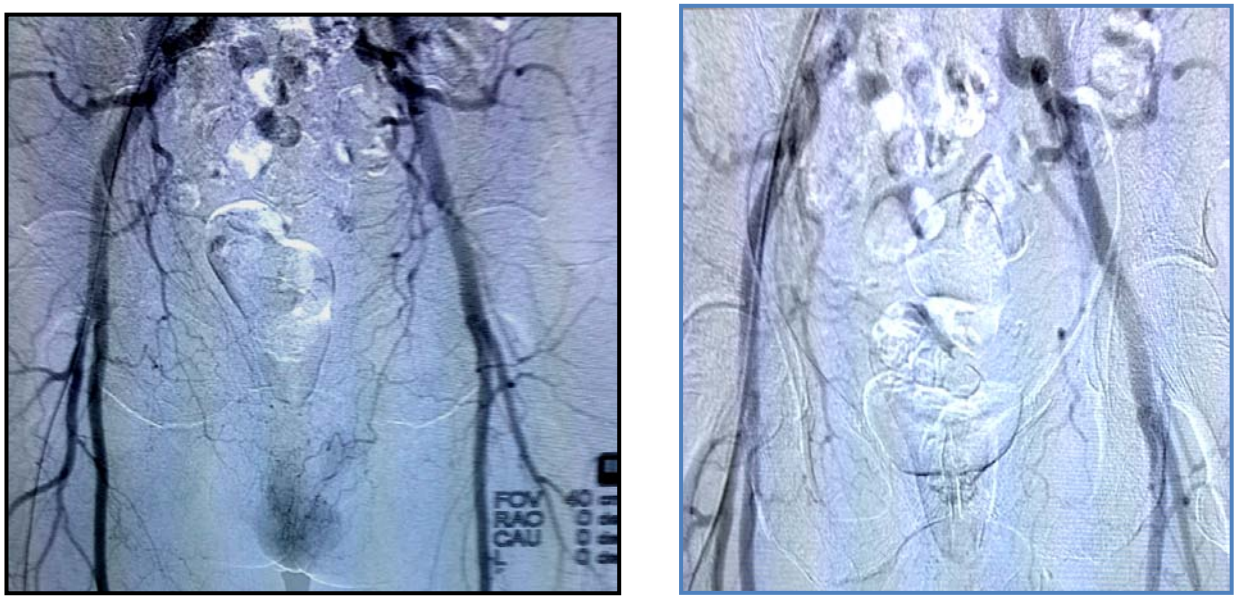


Fig. 3: Krioapplikatsiya and freezing the tumor.

In three patients with RV T3n2mo with tumor decay and bleeding, we undertook angiographic chemoembolization (HAE) with the introduction of 30 mg doxorubicin (Fig. 4).

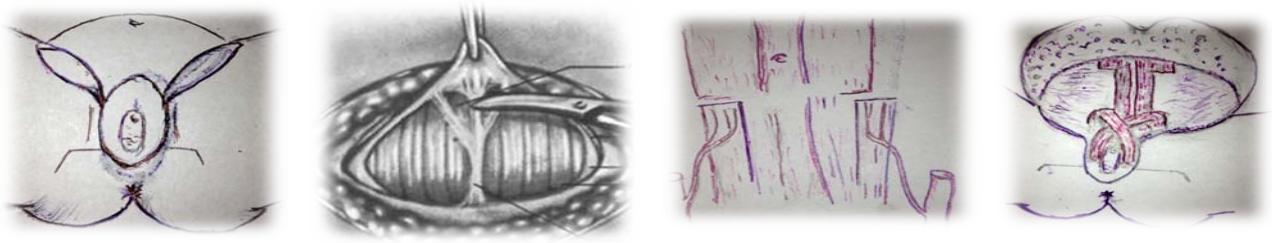


a) b)

Fig. 4: Angiographic chemoembolization a. pudenda interna: (a) angiography to HAE; b) angiography after HAE).

And although the diameter of a. pudenda interna did not allow to hold the catheter to the focus of pathology. Partial HAE it possible to reduce bleeding, restore hemoglobin by additional hemotransfusion and perform expanded vulvectomy and bilateral lymph node dissection.

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a) b) c) d)

Fig. 5: (a) excision of the vulva and inguinal areas; (b) and (c) Pfannenstiel incision exposing the rectus muscles and (a) epigastrica inferior; (d) formation of the labia majora by cross-segments of the rectus muscles.

With the aim of forming the volume of the external genitalia, improve the appearance quality and reduce lymphorrhea recently we have developed a method rekonstruktivnoi plastics (Patent No. 2580665 [4]) of the external genitalia through the mobilization of abdominal skin and fascial flap and the lower segments of the rectus muscles along with a.epigastrica inferior (Fig 5 a, b, c and d) using the apparatus "Harmonics". Closure of the wound was carried out at the beginning cross mobilized segments of direct muscles of a stomach on the vascular pedicle. The latter create the missing volume of the labia majora and represent a unique plastic material for vascularization and lymph drainage. In the area of the intersection of muscles and ends of the segment, fixation to the muscles of the vagina was performed by dissolving seams so that they did not hang over the mouth of the urethra and did not close the symphysis of the pubis. The next stage was sutured fascia of the rectus muscles of the abdominal wall. Then, 6-7 stitches were applied along the Donati to the skin of the perineum and the back wall of the vagina without tension in order to determine to what level the skin defect will be filled with an abdominal skin-fascial flap without tension.

Abdominal skin-fascial flap was placed on the wound surface, adapting it by cutting off excess and sharp skin areas, sutures were applied. Determining the projection of the abdominal flap, adjacent to the pubis, imposes two provisory internal anchor sutures to the periosteum, which is then stitched to the abdominal flap (without skin), genital forming a fold. Then through a separate puncture of the abdominal flap in the inguinal areas has introduced an active drainage in the inguinal-femoral area. Then every 0.8 cm for the tightness of stitches on the skin and the vaginal mucosa around the entire circumference and nodal skin sutures for Donati.

The operations were performed in 12 women, mostly aged 45-55 years with abdominal obesity. The process was localized on the skin and mucous membrane of the anterior half of the vulva. In one case, focal leukoplakia was an independent disease, in another case, an initial cancer was diagnosed. Healing took place by primary tension, except for 1 woman (8.3±1.4%) with obesity and type II diabetes mellitus. An important aspect of reconstructive vulvectomies by abdominal flap in combination with segments of straight muscles on the vascular pedicle was a decrease in the duration of lymphorhea for 2-3 days and the formation of the appearance of the organ.

IV. Discussion and Conclusions

As a result of the work carried out, mortality from cV (Fig. 6), despite the increase in morbidity, since 2005, when plastic surgery was introduced and modern approaches to tumor devitalization decreased by 6.2 times.

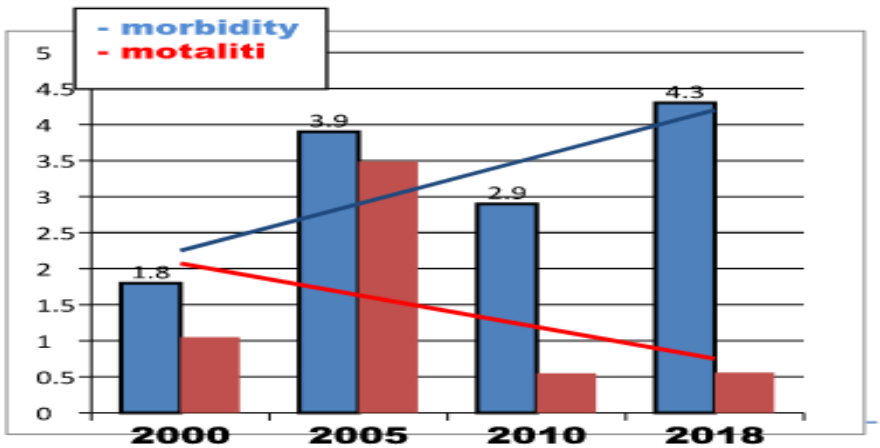


Fig. 6: Morbidity and mortality from cancer of the vulva (100 000 female population) in Novgorod region.

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Thus, further ways to improve the results of treatment lie, on the one hand, on the application and improvement of modern technologies, in particular PDT, cryodevitalization, apparatus "Harmonics" and reconstructive plastic surgery, and on the other, to improve cancer literacy of the population and General practitioners.

The use of reconstructive plastic operations in particular with the use of vascularized lower the segments of the rectus abdominis muscle is an individual method choice in young women with cancer of the vulva and vulgar dystrophy (VIN II-III), a measure of prevention of invasive forms cancer, helps to reduce complications, increase cosmetic effect and reduce the duration of lymphorrhea on average for 3-4 days.

Requires further study of the possibilities of the use of angiohemoembolization in neoadjuvant mode.

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Murray-Jackson-Lawler Syndrome

By Varsha Verma, Sumit Kar, Nidhi Yadav, Pooja Bonde, Pooja Manwar, Komal Ramteke & Safa Patrick

Mahatma Gandhi Institute of Medical Sciences

Abstract- Pachyonychia congenita (PC) is a rare autosomal dominant genodermatosis. It is of four types, type I due to mutation in genes 6a and 16, and 6b and 17 in type II. A 2 yr old male child presented in our OPD with hypertrophy of nails, hyperkeratotic papules over body, lusterless and sparse hair and natal teeth since childhood. Microscopy nail clippings and scrapping were done to rule out fungal infection. No evidence of any associated malignancy was found after thorough workup. He was diagnosed as PC Type 2. This case is being reported because of its rarity.

Keywords: pachyonychia congenita, genodermatosis, autosomal dominant, subungual hyperkeratosis, hyperkeratotic papules.

GJMR-F Classification: NLMC Code: WR 20



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Murray-Jackson-Lawler Syndrome

Varsha Verma [α], Sumit Kar [σ], Nidhi Yadav [ρ], Pooja Bonde [ω], Pooja Manwar [¥], Komal Ramteke [§] & Safa Patrick [χ]

Abstract- **Pachyonychia congenita (PC) is a rare autosomal dominant genodermatosis. It is of four types, type I due to mutation in genes 6a and 16, and 6b and 17 in type II. A 2 yr old male child presented in our OPD with hypertrophy of nails, hyperkeratotic papules over body, lusterless and sparse hair and natal teeth since childhood. Microscopy nail clippings and scrapping were done to rule out fungal infection. No evidence of any associated malignancy was found after thorough workup. He was diagnosed as PC Type 2. This case is being reported because of its rarity.**

Keywords: pachyonychia congenita, genodermatosis, autosomal dominant, subungual hyperkeratosis, hyperkeratotic papules.

I. Introduction

Pachyonychia congenital (PC) is a rare genodermatosis having autosomal dominant pattern of inheritance with high penetration. PC mainly affects a number of ectodermal structures including nail bed, skin, teeth, oral mucosa and pilosebaceous unit. Nail involvement is commonest feature of PC which is usually present at birth or develops soon after birth. Muller and Wilson described the first case of PC[1]. On the basis of clinical features found in addition to the nail changes, PC has been classified into four types. Here, we report a rare case of PC type-2, known as Murray-Jackson-Lawler syndrome.

II. Case Report

A 2-year-old male child born of a nonconsanguinous marriage presented with thickened, yellowish brown discoloration of all the nails and multiple papules all over his body and ill formed lower incisor since birth [Figure 1]. Initially, at the time of birth, parents noticed natal tooth (ill formed lower incisor) and thickening of few nails of toes and fingers, which gradually increased over months and involved total twenty nails [Figure 2 a, b]. Later, at the age of 3 months parents also noticed small hyperkeratotic papules over both knees [Figure 3], which over a period of year increased and involved bilateral upper limb, bilateral lower limb, trunk and buttocks. On examination, the nails showed thickened, lusterless nail plate of all the nails with upward growth of its distal portion. The nails also showed subungual hyperkeratosis and the nail plate was adhered to the underlying nail bed. There was increased curvature of the transverse axis of all nail plates giving a "pinched shape" to the free edge of the nail plate. Multiple hyperkeratotic follicular papules were present over bilateral upper limb, trunk, lower limbs and buttocks. He also had lusterless, sparse hair over scalp [Figure 4]. Systemic examination revealed no abnormality. The routine investigations were within the normal limits. Skin scraping for potassium hydroxide mount was negative for fungal elements. Patient's throat, and ophthalmological examination were normal. With all these findings the patient was diagnosed as PC type-2 and was started on topical retinoid and emollient for 1 month.

III. Discussion

Pachyonychia congenita is a rare group of disorder characterized by inherited ectodermal dysplasias transmitted in an autosomal dominant fashion. Some autosomal recessive cases also detected[2]. Sporadic case has been also detected with spontaneous mutation[3]. Pachyonychia congenita have four variants identified depending on the genetic mutation and clinical correlation[3]. (1) PC type-1 (Jadassohn Lewandowsky Syndrome) occurs because of mutations of the genes encoding keratin 6a and 16, characterized by subungual hyperkeartosis with dystrophic nail, focal palmoplantar keratoderma and follicular keratotic papules over body, (2) PC type-2 (Murray–Jackson–Lawler syndrome) occurs because of mutations of the genes encoding keratin 6b and 17, characterized by subungual hyperkeratosis with dystrophic nail, mild palmoplantar keratoderma, follicular keratotic papules, natal teeth and steatocystoma multiplex, (3) PC type-3 (Schafer–Branauer syndrome) characterized by subungual hyperkeratosis with dystrophic nail, focal palmoplantar keratoderma and follicular keratotic papules over body with angular cheilitis, corneal dyskeratosis, and cataracts, (4) PC type-4 characterized by subungual hyperkeratosis with dystrophic nail, focal palmoplantar keratoderma and follicular keratotic papules over body with laryngeal lesions, hoarseness of voice with mental retardation, hair abnormalities and alopecia. PC with late

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age of onset has been reported and termed as PC tarda [4]. The majority of mutations are missense mutations with a smaller number of deletions, insertions and splice site mutations in affected genes which leads to deleterious effects on protein structure as it interferes with the assembly of polypeptides forming the keratin structure of epidermal cell[5].

The main characterstic symptom of this syndrome is hyperkeratosis of the nail bed. This type of subungual hyperkeratosis leads to the elevation and increased transverse curvature of the nail plate and also associated with discoloration, thickening and friability of nail plate[6], which may sometimes fail to reach the distal fingertip. Generally all the 20 nails are affected but more severely thumbs, index fingers and toes nails are involved[7].

The treatment in this syndrome is usually unsatisfactory. Topical application of salicylic acid, urea and 5-fluorouracil can be advice to the patient. The other treatment modalities include systemic therapy like oral retinoids (acitretin, retinoic acid) and surgery[8]. A long term use of retinoids may results in to some degree of flattening of the nails and other complications such as periosteal hyperostosis, increased sensitivity and fragility of the underlying epidermis, and this limits their usefulness. [9] A genetic counselor also plays an important role as this gene has an autosomal dominant inheritance pattern and that PC can affect one half of his or her progeny, so counselor should inform the carrier. Nail findings persist for long, but other features may become less severe later in life.

Our patient belongs to PC type-2 as he had classical nail deformity along with hyperkeratotic papules over bilateral upper limb, bilateral lower limb, trunk and buttocks with natal teeth, and sparse lusterless thin hair over scalp. The patient did not show any of the other findings associated with other types of PC. This case is being reported because of its rarity.



Figure 1: Ill formed lower incisor since birth.



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Figure 2 (a,b): Yellowish Brown discoloration of nails and thickening of all twenty nails.



Figure 3: Small hyperkeratotic papules over both knees.

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Figure 4: Lusterless, sparse hair over scalp.

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Association between Body Mass Index and Diabetic Complications among Type-2 Diabetic Patients in Semi-Urban Area, Bangladesh

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Abstract- *Aims:* The purpose of the study was to estimate the association between body mass index and diabetic complications of patients with type 2 diabetes mellitus in the semi-urban area (Savar), Bangladesh.

Methods: A cross-sectional study was conducted among 420 type-2 diabetic patients, picked up conveniently from Jahangirnagar University and nearest community, Savar, Dhaka. Body mass index (BMI) was calculated and categorized according to The WHO recommended Asian criteria. Diabetic complications were identified by clinical signs and symptoms using a questionnaire. Multiple logistic regression and Chi-square test were employed in data analysis.

Keywords: *type-2 diabetes; BMI; hypertension; nephropathy; neuropathy.*

GJMR-F Classification: *NLMC Code: WK 810*



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Association between Body Mass Index and Diabetic Complications among Type-2 Diabetic Patients in Semi-Urban Area, Bangladesh

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Abstract- Aims: The purpose of the study was to estimate the association between body mass index and diabetic complications of patients with type 2 diabetes mellitus in the semi-urban area (Savar), Bangladesh.

Methods: A cross-sectional study was conducted among 420 type-2 diabetic patients, picked up conveniently from Jahangirnagar University and nearest community, Savar, Dhaka. Body mass index (BMI) was calculated and categorized according to The WHO recommended Asian criteria. Diabetic complications were identified by clinical signs and symptoms using a questionnaire. Multiple logistic regression and Chi-square test were employed in data analysis.

Results: A total of 420 type-2 diabetic patients were investigated. Out of these, 248 were male, and 172 were female (mean±SD of age, 47.5±6.4 years). It was estimated that 23.8% had normal weight, 44.8% were overweight, and 31.4% were obese. The study found significant association between body mass index (BMI) and complications faced by the patients: BMI and hypertension (X^2=14.987, df =2, p=0.001); BMI and neuropathy (X^2=14.697, df=2, p=0.001); BMI and retinopathy (X^2=9.412, df=2, p=0.009); BMI and nephropathy (X^2=25.503, df=2, p=0.000). Multiple logistic regressions indicated that overweight and obese were significant predictors of diabetic complications (P<0.05).

Conclusion: Globally, the prevalence of diabetes and overweight/obese have been increasing rapidly. People who are either overweight or obese accompanied by diabetes are at increased risk of developing complications. Therefore, weight control may reduce the risk of developing diabetes and its complications.

Keywords: type-2 diabetes; BMI; hypertension; nephropathy; neuropathy.

I. Introduction

Diabetes has become a rapidly growing health burden worldwide.[1] American Diabetes Association (ADA) has defined Diabetes Mellitus(DM) as, "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both."[2] DM is a disease known since ancient times, cited by both the Greeks and Egyptians in as early as 4,500 BC.[3] An estimated 285 million people have type-2 diabetes globally, making up about 90 percent of all diabetes cases.[4] In the South Asian region, Bangladesh has the second largest number of diabetes patients with a prevalence rate of 11%.[5]

Almost all of the complications of diabetes are caused by having too much blood glucose.[6] Type 2 diabetes mellitus (T2DM) and it's macro- and micro-vascular complications are a major threat to global public health.[7] The Centers for Disease Control and Prevention (CDC) reported that adults with diabetes are three times more likely than those without diabetes to have a history of coronary artery disease, three times more likely to have a stroke, and two times more likely to have another heart condition.[8] It is one of the major causes of premature illness and death in most countries worldwide. The World Health Organization (WHO) reported that diabetes was the seventh leading cause of death and contributed to an estimated 1.6 million deaths in 2016.[9]Complications Like premature atherosclerotic cardiovascular diseases, nephropathy leading to renal failure and peripheral neuropathy, cardiovascular symptoms. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.[10]

Overweight and obesity are driving the global diabetes epidemic. They affect the majority of adults in most developed countries and are increasing rapidly in developing countries.[11] There are several studies indicating that weight loss and exercise may help in the treatment of diabetes. Weight loss and exercise have both been shown to decrease insulin resistance, a major physiological defect related to the development of diabetes, and to improve glycemic control. These interventions also ameliorate hypertension and lipid abnormalities and thus may contribute to a reduction in risk of coronary heart disease in individuals with T2DM.[12] Hypertension is a common problem in diabetic patients. Markedly increases the risk and accelerates the course of cardiac disease, peripheral vascular disease, stroke, and nephropathy. Hypertension is approximately twice as frequent in patients with diabetes compared with patients without the disease.[13]

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The significance of the study is derived from the available statistics that reflect a high prevalence of DM in Bangladesh; more than 11 % and it will be increased 13% by 2030.[14] These huge number of people suffering from many kinds of diabetic complications which causes an increase in health care expenditures. So there is a need for research to reduce the diabetic morbidity. Moreover, to our knowledge, there is no study in a semi-urban area in Bangladesh that estimates of the association between a range of diabetes complications and Body Mass Index (BMI). Therefore, the current study aimed to estimate the association between diabetic complications and BMI at Jahangirnagar University and nearest Community, Savar, Bangladesh.

II. Materials and Methods

a) Study design and population

It was a cross-sectional study conducted among diagnosed type-2 diabetic patients at Jahangirnagar University and the nearest community, Savar, Dhaka. The study was carried out in the year 2017 and 2018. A total of 420 patients with type-2 diabetes were selected conveniently. The sample size was determined by using the formula, $n=z^2pq/d^2$.

b) Data collection

Before collection, informed consent was taken from each patient. The purpose of the interview was clearly explained to the study subjects. A semi-structured questionnaire was used as the research instrument. Data were collected by face to face interview.

c) Measures

Weight, height, and blood pressure were measured by using valid equipment. This study adopted the BMI definition of the World Health Organization (2000).[15] Body weight was measured to the nearest 100 gm. A professional weight machine was used for body weight measurement. The height was measured in centimeter by using a measuring tape. Blood pressure (systolic and diastolic) recordings were made after the subjects had rested in the sitting position for 10 minutes using a validated sphygmomanometer. Three separate readings were taken, and their mean was recorded.[16] Nephropathy, neuropathy, and retinopathy were diagnosed by clinical signs and symptoms using questionnaire.

d) Data Analysis

Data were collected and analyzed using Statistical Package for Social Science (SPSS), version 20.0. Chi-square test was employed to test the association between variables. Linear multiple logistic regression was employed to identify predictors of diabetic complications. The statistically significant result means that the P-value is less or equal to 0.05.

III. Results

A total of 420 patients with type-2 diabetes mellitus (T2DM) were interviewed in the present study. The basic socio-demographic characteristics of the patients were shown in Table-1. The mean BMI of the patients was 27.37±5.04 Kg/m^2. According to the BMI category, out of 420 patients, 23.8% had normal weight, 44.8% were overweight, and 31.4% were obese. (Table-1)

During the study period, blood pressure of the patients was measured. About 20% of the patients were found to be hypertensive. The patients were also asked about the complications they faced due to diabetes. The study found that 18.1% had diabetic neuropathy, 19.0% had diabetic nephropathy, and 21.9% of patients had diabetic retinopathy. (Table-2)

The study found significant association between body mass index (BMI) and complications faced by the patients: BMI and hypertension (X^2=14.987, df =2, p=0.001); BMI and neuropathy (X^2=14.697, df=2, p=0.001); BMI and retinopathy(X^2=9.412, df=2, p=0.009); BMI and nephropathy (X^2=25.503, df=2, p=0.000). (Table-3)

Logistic regression analysis further revealed that BMI was a significant predictor of complications arise among the patients with T2DM (p<.05). Obese patients were 3.3 times more (OR=3.3, 95% CI=1.629-6.699; p=0.001) and overweight patients were 1.5 times more (OR=1.5, 95% CI=0.737-3.069; p=0.001) likely to develop hypertension than patients with normal weight. Similarly, obese patients were at more risk to develop neuropathy than patients with normal weight (OR=2.968, 95% CI=1.516-5.811; p=0.001). Patients who were overweight were more prone to develop retinopathy than those who were normal (OR=2.804, 95% CI=1.417-5.549; p=0.011). The results also show that patients who were overweight were more likely to develop nephropathy than patients with normal weight (OR=1.073, 95% CI=0.512-2.249; p=0.000). Similarly, patients who were obese were about four times more likely to develop nephropathy than patients with normal weight (OR=3.667, 95% CI=1.815-7.409; p=0.000). (Table-4)

IV. Discussion

Diabetes mellitus is a leading contributor to death and disability worldwide. The prevalence of diabetes and overweight/obese have increased rapidly worldwide.[18, 19] Similarly, the prevalence of diabetes and the percentage of people either overweight or obese have increased substantially in Bangladesh.[19] To our knowledge, there is no study in a semi-urban area in Bangladesh that estimates of the association between a range of diabetes complications and BMI. The present study provided the opportunity to estimate of the association between diabetic complications and BMI

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among Jahangirnagar University community and nearest community in Savar, Bangladesh.

This study found that more or less twenty percent of the patients had a range of diabetic complications, including hypertension, neuropathy, retinopathy, and nephropathy. Diabetic patients with long term uncontrolled blood sugar may develop various serious complications. Among the most prevalent complications are kidney disease, blindness, and amputations reported in previous studies. [20, 21] Elevated blood pressure is observed in diabetic patients about 1.5 to 2 times more frequently than among non-diabetic patients. [22] The study estimated that almost half of the diabetic patients were overweight, whereas one-third of those were obese. Physical inactivity and patient's reluctances to follow dietary guideline may be the reasons for weight gain in Bangladesh.

Overweight and obesity are known to increase blood pressure, which is the leading cause of strokes. Excessive weight gain also increases the chances of developing other problems, including high cholesterol, high blood sugar, and heart disease.[23] Diabetic patients, either overweight or obese, are at increased risk of developing diabetic complications. Obesity accompanying with T2DM is known to be closely linked with insulin resistance and elevated sympathetic nervous activity.[24] It has been frequently reported in the literature that obesity, hypertension, and diabetes are high-risk factors for subsequent cardiovascular and renal complications. This study revealed that hypertension, neuropathy, retinopathy, and nephropathy were associated with BMI ($p<0.05$). More or less comparable results were reported in some previous studies.[22, 24, 25] Weight control is an important step for the management of diabetic complications. Physical activity helps to maintain a healthy weight. Regular exercise may bring about many possible health benefits, and contribute to weight loss, prevent weight regain, improve insulin sensitivity, glycaemic control.[26]

V. Conclusion

Globally the prevalence of diabetes and overweight/obese have been increasing rapidly. People who are either overweight or obese accompanied by diabetes are at increased risk of developing complications. Therefore, weight control may reduce the risk of developing diabetes and its complications.

VI. Declarations

Funding

Self.

Ethical issues

This study was conducted maintaining ethical standards to the highest possible extent. Before the assessment, informed consent was taken from all the patients, participated in this study. This study was approved by the Department of Public Health and Informatics, Jahangirnagar University, Savar, Dhaka. The study was also followed by "recommendations guiding physicians in biomedical research involving human subjects," adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964.

Conflict of interest

No conflict of interest.

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Limitations

The study was not free of limitation. The population was selected conveniently, so there might be a chance of bias. Limited sample size due to self-funding may restrict for generalization. The limited resources such as reports, statistical data, books, and journals were also a limitation.

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Table 1: Socio-demographic characteristics of the patients with T2DM (n=420)

Variables	Frequency	Percentage (%)	Mean(±SD)
Socio-demographics			
Gender			
Male	248	59.0	
Female	172	41.0	
Age (years)			
30-40	60	14.3	47.2(±6.4)
40-50	204	48.6	
>50	156	37.1	
Education			
Low educated (1-9)	224	53.3	
High educated (>9)	196	46.7	
Employment status			
Unemployed	144	34.3	
Employed	276	65.7	
Marital status			
Unmarried	12	2.9	

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Married	408	97.1	
Monthly income (BDT)			
5,000-15,000	116	27.6	25,305 (±11,507)
15,000-30,000	208	49.5	
>30,000	96	22.9	

Table 2: Complications reported by the patients with T2DM (n=420)

Complications	Frequency	Percentage (%)
Hypertension		
Yes	85	20.2
No	335	79.8
Neuropathy		
Yes	90	21.4
No	330	78.6
Retinopathy		
Yes	92	21.9
No	328	78.1
Nephropathy		
Yes	80	19.0
No	340	81.0

Table 3: Distribution of various complications of the patients with T2DM according to BMI category (n=420)

Variables	Total (420); n (%)	Complications; n (%)	X^2 test value	df	p-value
BMI		**Hypertension**			
Normal	100(23.8)	12 (12.0%)	14.987	2	.001
Overweight	188(44.8)	32(17.0)			
Obese	132(31.4)	41(31.1%)			
BMI		**Neuropathy**			
Normal	100(23.8)	14(14.0%)	14.697	2	.001
Overweight	188(44.8)	33(17.6%)			
Obese	132(31.4)	43(32.6%)			
BMI		**Retinopathy**			
Normal	100(23.8)	12(12.0%)	9.412	2	.009
Overweight	188(44.8)	52(27.7%)			
Obese	132(31.4)	28(21.2%)			
BMI		**Nephropathy**			
Normal	100(23.8)	12(12.0%)	25.503	2	.000
Overweight	188(44.8)	24(12.8%)			
Obese	132(31.4)	44(33.3%)			

Table 4: Association of BMI category with various complications of the patients with T2DM as explored by binary logistic regression (n =420)

Variables	Unadjusted Model		
	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
BMI	**Hypertension**		
Normal	Ref		.001
Overweight	1.504	(0.737-3.069)	
Obese	3.304	(1.629-6.699)	
BMI	**Neuropathy**		
Normal	Ref		.001
Overweight	1.308	(0.664-2.577)	
Obese	2.968	(1.516-5.811)	
BMI	**Retinopathy**		
Normal	Ref		.011
Overweight	2.804	(1.417-5.549)	
Obese	1.974	(0.948-4.111)	
BMI	**Nephropathy**		
Normal	Ref		.000
Overweight	1.073	(0.512-2.249)	
Obese	3.667	(1.815-7.409)	

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Functional Dietary Supplementation of Okara (Soybeans Residue) on Streptozotocin Induced Diabetes Mellitus in Male Wistar Rats

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Keywords: *diabetes mellitus, liver function, okara diet, lipid profile, and glycated hemoglobin.*

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Functional Dietary Supplementation of Okara (Soybeans Residue) on Streptozotocin Induced Diabetes Mellitus in Male Wistar Rats

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Abstract- A poor dietary habit has been demonstrated to be one of the key players in the development of diabetes mellitus, and a diet rich in dietary fiber has been highlighted to be a potent candidate for the management of diabetes mellitus. Therefore, this study is aimed to validate the role of dietary supplementation of okara (soybeans residue) in streptozotocin induced diabetic male Wistar rats. The total of 28 rats between the weight of 100 to 105g, was grouped into four n=7, and this study spanned for a period of 43days. All experimentations were conducted using standard method, and our findings show that the cumulative feed intake of 15% okara diet supplementation was significantly higher $p<0.05$ particularly from day 29 to day 43 relative to the negative control. After treatment for a period of 43days, 6mg/kg glibenclamide treated group 226.33±6.38 and 15% okara supplemented diet fed group 219.83±5.67 showed a significant increase $p<0.05$ in body weight relative to the Negative control 161.17±3.60. 15% okara diet supplementation significantly lowered $p<0.05$ blood sugar levels after treatment relative to after induction similar to 6mg/kg glibenclamide treated group. Glycated hemoglobin, glucose-6-phosphate dehydrogenase, lipid profile (CHOL, TRIG, LDL and HDL), liver function enzymes (AST, ALT, ALP and GGT), kidney function biomarkers (Creatinine, Urea, Sodium and potassium) and antioxidant enzymes (CAT, SOD, GSH, GST and GPX) were all significantly restored within the normal range. Histological observations of the pancreatic, liver, and kidney tissue showed no visible lesion for 15% Okara supplemented diet feeding. Conclusively, we recommend food supplementation with Okara.

Keywords: diabetes mellitus, liver function, okara diet, lipid profile, and glycated hemoglobin.

I. Introduction

Diabetes mellitus (DM) is a metabolic disorder whereby either the pancreas does not produce enough insulin (a hormone that regulates hyperglycemia), or situation where the body cannot effectively use the insulin it produced [1]. Correspondingly, DM has not only assumed a pandemic proportions worldwide but has also proven to affect the developing countries of the world much more than their developed counterparts [2]. Of course, DM has been demonstrated to be a prime global health concern with a projected rise in prevalence from 171 million in 2010 to 366 million in 2030 [1]. Disturbingly, both the number of cases and the prevalence of DM has steadily been showed to be on the rise over the past few decades, and it is regarded to be a silent killer disease, affecting millions of peoples in the world [3, 4]. Considering Africa, studies has revealed that, the number of people with diabetes will increase from 14.2 million in 2015 to 34.2 million in 2040 with majority of the cases predominated in some of the region's most populous countries like: South Africa, the Democratic Republic of Congo, Nigeria, and Ethiopia [2, 3].

Regardless of the numerous conventional medications that have been in use for the management of DM, it inaccessibility has been a limitation as a result of the relatively high cost and sometimes unavailability [5]. In this light, of course, a switch to a readily available and cheaper alternative has become necessary in the form of phyto/herbal medicine [5]. Herbal medicine similarly referred as phytomedicine; alludes to the use of plants seeds, flowers, roots for medicinal purpose and even today, plant materials continue to play an important role in primary health care as a therapeutic remedy in many developing countries [5]. As reported, the World Health Organization (WHO) recently recommended the use of medicinal plants for the management of DM and further encouraged the expansion of the frontiers for scientific evaluation on the hypoglycemic properties of diverse plant species [5,6].

Soybean-based foods have shown to be beneficial on human health and currently the consumption of soybean products elevated due to functional food improving knowledge [7,8]. Generally, it has been demonstrated that a diet high in fiber is useful in the management of the plasma glucose concentration in individuals with diabetes [9]. The beneficial metabolic effects of dietary fiber are long lasting and clinically relevant both in types 1 and type 2 diabetic patients [10]. Also, Fiber has been studied in the treatment of diabetes for many years because increased fiber content has been shown to decrease the glycemic index of foods. The theory, then, is that the decreased glycemic index would lead to smaller increases in blood glucose, and thus reduced blood glucose and HbA1c levels [11].

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Okara is the insoluble residue from soybean after milk production and is mainly rich in dietary fiber 50–60% and protein 30% [12]. Its dietary fiber has 12% hemicellulose, 5.6% cellulose, 12% lignin, and 0.16% phytic acid [13]. Because of its high fiber content, okara is used as a supplement in human diets, particularly western diets, which are deficient of the essential fiber [12].Although there have been several reports on the nutraceutical power of okara [7, 14, 15], still, the use is not yet a common practice, as most people still dispose of it in the form of chaff after extraction. In this light, continual evaluation and research are required to validate and update the existing report on the health benefit of okara. Therefore, the aim of this study was to investigate the antidiabetic ability of okara (soybean residue) onstreptozotocin induced diabetic male Wistar rats.

II. Material and Methods

a) Seed collection and preparation

Soybean Seeds were purchased from Bodija market, Ibadan, Oyo State. Thereafter, it was identified by a botanist in the Botany Department of the University of Ibadan. The seeds were sorted manually to remove defective seeds and other extraneous materials and then washed. The washed soybean seeds were blanched in hot water for 25minutes at 100°C and then dehulled. The dehulled cotyledons were washed with hot (100°C) water twice and wet milled using 5litres of water to 1kg of the soybean seeds. The slurry obtained was mixed and filtered through a muslin cloth to remove the milk and recover the residue called okara. The fresh okara was dried using a hot-air dryer at a temperature of 70°C, milled and sieved through 0.25mm pore sized sieve. Okara flour was then packaged hermetically and stored for analyses and diet formulation.

b) Analysis of feed

We carried out a proximate content analysis for protein, fat, ash, fiber and moisture according to a standard procedure as described by [16], and mineral (calcium and phosphorus) content analyses were carried out using Atomic Absorption Spectrophotometry (AAS).

Table 1: Feed Composition

Parameters	Normal Diet (%)	Okara Diet (%)
Protein	21	24.5
Ash	1.3	4.2
Moisture	4.6	7.4
Fat	3.5	3.2
Fibre	6.0	40.8
Calcium	0.8	0.9
Phosphorus	0.8	0.72
Okara	-	15
Dry Matter	-	91.38%

c) Chemical

All the chemicals used in this study were of analytical grade unless stated otherwise. Streptozotocin (Santa Cruz, U.S.A), Potassium chloride (BDH Chemical Ltd, England), dipotassium hydrogen phosphate K_2HPO_4 (Hopkins and Williams Ltd. England) and anhydrous potassium dihydrogen phosphate KH_2PO4 (BDH Chemical Ltd. England)

d) Animals used

For this study, a total of 28 male Wistar rats between the weights of 100-105g were procured from the central animal house, College of Medicine, University of Ibadan, Nigeria. The male Wistar rats were kept in well-kempt and ventilated cages, and their beddings changed every three days, and were allowed free access to clean drinking water. The rats were allowed to acclimatize for two weeks before commencement of experimentation, and all the processes involved in handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the department.

e) Induction of Hyperglycemia with Streptozotocin

Hyperglycaemia was induced with a single dose of intraperitoneal injection of streptozotocin. 50 mg/kg of streptozotocin was dissolved in (0.1 M, pH 4.5) citrate buffer and after 72 hours; ACCU-CHEK Glucometer was used to measure blood sugar level from blood samples collected from a caudal vein of the rats. Blood sugar levels between the values of (326.50±3.56 to 327.00±4.85 mg/dl) were taken to be diabetic and the values in the range of (88.67±2.16 to 97.67±1.51 mg/dl) were taken to be normal in this study.

f) Measurements of food intake and body weight

Daily food intake was derived by (final feed weight – initial feed weight) on a daily basis throughout the experimentation, and body weight was taken weekly with an electronic balance.

g) Experimental design

Each group contained seven animals.

Group 1: Normal Control fed with a normal diet.

Group 2: Negative control received 50mg/kg STZ, fed a normal diet and remained untreated.

Group 3: Positive control received 50mg/kg STZ, fed normal diet, and treated with glibenclamide 6mg/kg as used by [17].

Group 4: Received 50mg/kg STZ, fed 15% okara supplemented diet as used by [7].

h) Biochemical analysis

ACCU-CHEK glucometer was used to measure blood sugar level, glucose-6-phosphate dehydrogenase (G6PD) was measured by spectrophotometric method using Randoxkits, Randox kit method of enzymatic hydrolysis described by [18] was used for determination

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of triglyceride, total cholesterol, and high-density lipoprotein-cholesterol. Glycated hemoglobin (HBA1c) was measured at the end of the study using a high-performance liquid chromatography (HPLC) technique. Serum level of alanine and aspartate aminotransferases (ALT and AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatinine, urea, sodium, and potassium levels were measured by spectrophotometric method using Randox kits. Superoxide dismutase (SOD) activity was measured using the method of [19] as previously described by [1], catalase (CAT) activity was measured using the method of [20] as previously described by [1], reduced glutathione (GSH) concentration was estimated using the method of [21] as previously described by [1], Glutathione-S-transferase activity was determined following the method of [22] as previously described by [1], and Glutathione peroxidase (GPX) activity was estimated following the method of [23].

i) Histopathological Studies

Small pieces of the pancreatic, liver, and kidney tissues were fixed in 10% formalin solution, followed by embedding in melted paraffin wax. Histopathological assessment and photomicrography of the prepared slides were done by using an Olympus light Microscope with attached Kodak digital camera as previously described by [1].

j) Statistical analysis

As previously reported in [1], data were analyzed using ANOVA (analysis of variance) and mean separation was done using Duncan multiple range test and HSD Turkey. Paired T-test was used to establish a difference in timely events. P values less than 0.05 ($p<0.05$) were considered significant. Data were expressed as means ± standard deviation and pictorially presented in the form of charts. All statistical analysis was done using IBM SPSS Version 22 and Microsoft Excel.

III. Results

a) Feed intake

Table 2 shows the cumulative feed intake of male Wistar rats. Following two weeks of acclimatization, the weight of the feed consumed by each group was recorded on a daily basis, summed up and reported weekly as day 1, day 8, day 15, day 22, day 29, day 36 and day 43 respectively. On day 1, there was no significant difference $p>0.05$ in the amount of feed consumed between all the groups. However, the experimental groups (B, C, and D) intraperitoneally injected with 50mg/kg streptozotocin showed a significant increase $p<0.05$ in cumulative feed intake relative to the normal control at day 8, day 15 and day 22 respectively. Interestingly, the cumulative feed intake from day 29 up till day 43 for 15% okara supplemented diet fed group was significantly different $p<0.05$ the negative control and showed no significant difference $p>0.05$ as compared to the normal control.

Table 2: Cumulative feed intake of male Wistar rats

G	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
A	24.8 ± 1.17^{a}	33.7 ± 1.03^{a}	50.3 ± 1.63^{a}	62.7 ± 5.61^{a}	96.3 ± 5.28^{a}	132.3 ± 1.51^{c}	174.8 ± 1.72^{c}
B	25.0 ± 1.41^{a}	42.2 ± 0.75^{b}	71.3 ± 1.63^{b}	93.3 ± 2.07^{b}	105.7 ± 0.82^{b}	119.2 ± 2.23^{a}	156.2 ± 1.17^{a}
C	24.5 ± 1.64^{a}	42.8 ± 1.47^{bc}	72.8 ± 2.04^{b}	92.8 ± 2.56^{b}	117.5 ± 1.52^{c}	128.7 ± 2.42^{b}	165.0 ± 2.10^{b}
D	24.7 ± 1.21^{a}	44.2 ± 1.47^{c}	73.5 ± 1.87^{b}	93.3 ± 1.63^{b}	125.7 ± 1.63^{d}	131.5 ± 1.52^{bc}	173.3 ± 3.27^{c}

Mean value with the same alphabet as superscript within each column variable are non-significant ($p>0.05$). The abbreviations represent G: Groups, A: Normal Control, B: Negative Control, C: Positive Control, D: 15% Okara Diet supplementation.

b) Body weight

Figure 1 demonstrates the effect of 15% okara diet on body weight (g). Following two (2) weeks of acclimatization, the rats body weights were taken and reported as initial body weight, and there was no observed significant difference $p>0.05$ between all groups. However, after the experimental groups received 50mg/kg streptozotocin intraperitoneal injection, the body weights were recorded after 7 days, and the result revealed that there was a significant reduction $p<0.05$ in body weight of all experimental groups; negative control 138.17±2.86, positive control 138.17±1.33 and 15% okara supplemented diet fed group 140.50±2.81 relative to the normal control 150.67±2.73. The final body weights of the rats were recorded after treatment for a period of 43 days, 6mg/kg glibenclamide treated group 226.33±6.38 and 15% okara supplemented diet fed group 219.83±5.67 showed a significant increase $p<0.05$ in body weight relative to the Negative control 161.17±3.60. Similarly, the normal control also showed a significant weight increase $p<0.05$ compared to the negative control.

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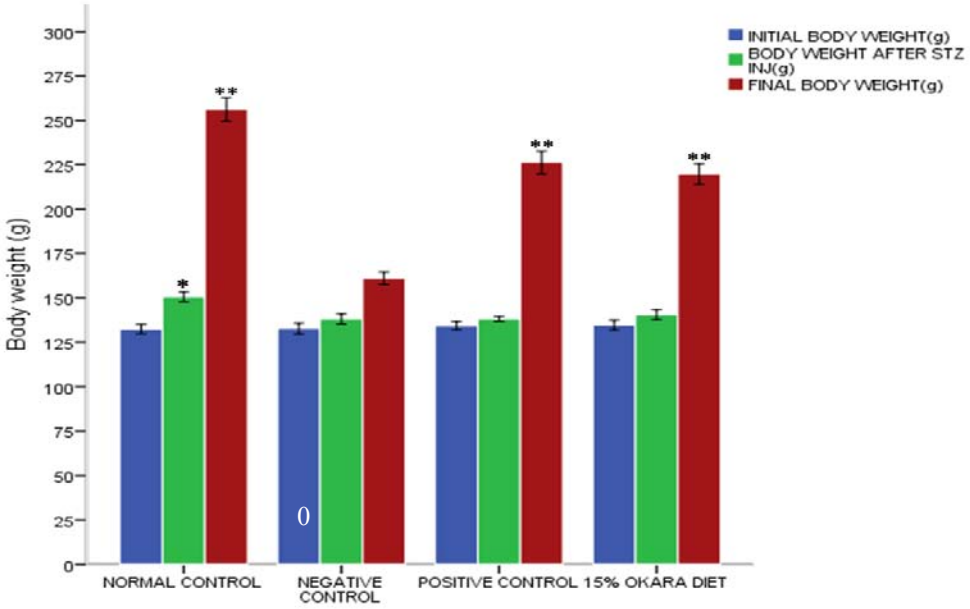


Figure 1: Effect of 15% Okara supplemented diet on body weight of male Wistar rats

* Significant (P<0.05) vs. Initial body weight
** Significant (P<0.05) vs. Body weight after streptozotocin injection

c) Blood sugar levels

Figure 2 shows the effect of 15% okara supplementation diet on blood sugar levels. Baseline blood sugar levels were recorded after two (2) weeks of acclimatization; Blood sugar levels after induction were recorded after 72 hours of intraperitoneal injection of 50mg/kg streptozotocin, and after treatment at end of 43days, blood sugar levels were also recorded. Normal control showed no significant variation p>0.05 in blood sugar levels, after treatment 97.67±1.51 relative to after induction 92.67±4.21, after induction relative to baseline 89.17±1.72. The experimental groups showed a significant elevation p<0.05 in blood sugar level after induction relative to the baseline. However, 15% okara diet supplementation and 6mg/kg glibenclamide treated group significantly lowered p<0.05 blood sugar levels after treatment relative to after induction.

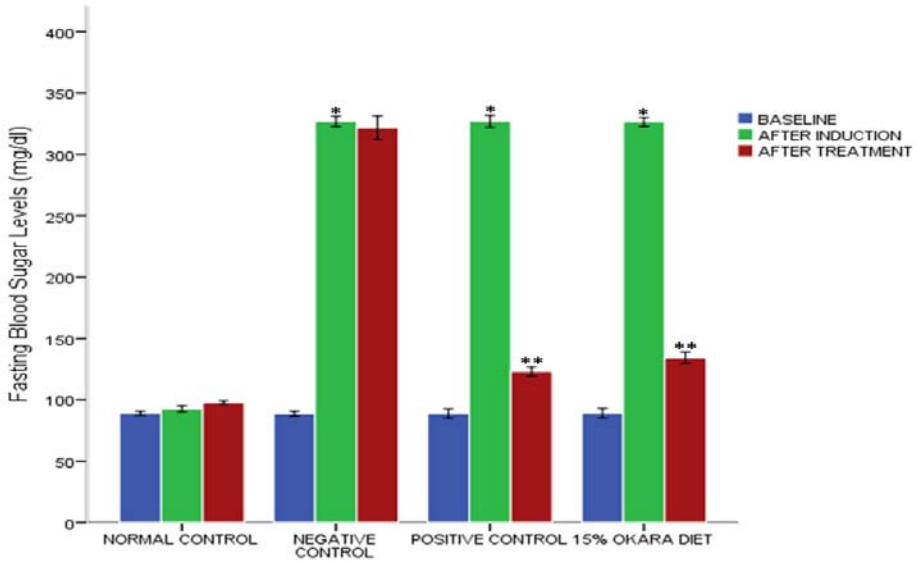


Figure 2: Effect of 15% Okara supplemented diet on blood sugar levels of male Wistar rats

* Significant (P<0.05) vs. Initial Baseline
** Significant (P<0.05) vs. Body after induction

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d) Glucose-6-phosphate dehydrogenase and glycated hemoglobin

Table 3 showed that G6PD ranged from (Normal Control) 91.33±4.32 to 46.00±2.83 (Negative Control-Untreated diabetics) and HBA1c ranged from (Normal Control) 4.62±0.28 to 11.28±0.54 (Negative Control-Untreated diabetics). In each parameter assay, 15% okara supplemented diet, 6mg/kg glibenclamide (positive control) treated group, and the normal control showed a significant increase $p<0.05$ and a significant decrease $p<0.05$ relative to the negative control.

Table 3: Effect of 15% okara supplemented diet on HBAlc and G6PD

GROUPS	G6PD	HBAlc %
Normal Control	91.33 ± 4.32^{d}	4.62 ± 0.28^{a}
Negative Control	46.00 ± 2.83^{a}	11.28 ± 0.54^{d}
Positive Control	74.50 ± 5.21^{c}	6.37 ± 0.36^{b}
15% Okara Diet	58.17 ± 2.56^{b}	7.57 ± 0.52^{c}

Mean value with the same alphabet as superscript within each column variable are non-significant ($p>0.05$). The abbreviations represent G6PD: glucose-6-phosphate dehydrogenase, HBAlc: Glycated hemoglobin.

e) Lipid Profile

Table 4 shows the effect of 15% okara supplemented diet on lipid profile. At the end of 43 days of treatment, blood samples were collected from the rats, and plasma levels of lipid profile parameters (CHOL, TRIG, LDL, and HDL) were determined. Negative control (untreated group) showed a significant elevation $p<0.05$ in the levels of CHOL, TRIG, LDL, and a significant decrease in the levels of HDL relative to the Normal control. However, the treated groups (15% okara diet and positive control) significantly lowered $p<0.05$ CHOL, TRIG, LDL, and elevated levels of HDL relative to the untreated group (Negative control).

Table 4: Effect of 15% okara supplemented diet on lipid profile

Groups	CHOL	TRIG	LDL	HDL
Normal Control	51.00 ± 1.55^{a}	34.83 ± 2.23^{a}	17.00 ± 1.67^{a}	40.00 ± 3.74^{c}
Negative Control	70.17 ± 1.17^{c}	51.67 ± 1.86^{d}	28.67 ± 1.63^{c}	21.83 ± 1.17^{a}
Positive Control	57.50 ± 3.39^{b}	41.00 ± 1.41^{b}	20.50 ± 1.87^{b}	30.17 ± 1.60^{b}
15% Okara Diet	56.17 ± 1.17^{b}	44.67 ± 1.75^{c}	22.17 ± 2.48^{b}	30.17 ± 2.14^{b}

Mean value with the same alphabet as superscript within each column variable are non-significant ($p>0.05$). The abbreviations represent CHOL: cholesterol, TRIG: triglyceride, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

f) Liver function

At the end of the experimentation, the rats blood samples were collected, and serum levels of liver function biomarkers ALT, AST, ALP, and GGT respectively were determined as demonstrated in table 5. Negative control which had remained untreated after 50mg/kg intraperitoneal streptozotocin injection showed a significant elevation $p<0.05$ in all liver biomarkers assayed for relative to the normal control. However, 15% okara supplemented diet and 6mg/kg glibenclamide significantly lowered $p<0.05$ levels of liver biomarkers vis-à-vis negative control.

Table 5: Effect of 15% okara supplemented diet on liver function

Groups	ALT(U/l)	AST(U/l)	ALP(U/l)	GGT(U/l)
Normal Control	32.33 ± 2.94^{a}	39.50 ± 2.34^{a}	125.67 ± 2.94^{a}	5.67 ± 1.21^{a}
Negative Control	44.67 ± 2.01^{b}	53.67 ± 2.34^{b}	164.17 ± 2.86^{b}	15.67 ± 1.63^{b}
Positive Control	58.83 ± 2.23^{c}	63.67 ± 2.94^{c}	143.25 ± 2.51^{c}	9.00 ± 0.89^{c}
15% Okara diet	44.00 ± 2.53^{b}	51.67 ± 1.86^{b}	144.33 ± 4.80^{b}	8.17 ± 0.98^{b}

Mean value with the same alphabet as superscript within each column variable are non-significant ($p>0.05$). The abbreviations represent ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma-Glutamyltransferase.

g) Kidney Function

Table 6 shows the effect of 15% okara supplemented diet in male Wistar rats. Serum levels of kidney function markers creatinine, urea, potassium, and sodium respectively were determined at the end of the experimentation. The experimental groups received 50mg/kg streptozotocin intraperitoneal injection. However, the negative control which remained untreated showed a significant increase $p<0.05$ in the levels of kidney function markers relative to the normal control and treatment with 15% okara supplemented diet feeding significantly $p<0.05$ lowered the levels in comparison with the negative control and of course similar with the positive control.

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Table 6: Effect of 15% okara supplemented diet on kidney function

Groups	Creatinine	Urea	Potassium	Sodium
Normal Control	51.67 ± 1.03^{a}	15.17 ± 1.17^{a}	6.50 ± 0.55^{a}	147.50 ± 2.17^{a}
Negative Control	70.00 ± 1.41^{c}	26.67 ± 1.37^{b}	10.67 ± 1.21^{c}	164.17 ± 3.00^{c}
Positive Control	53.00 ± 0.89^{a}	18.00 ± 1.72^{a}	8.83 ± 1.72^{b}	152.67 ± 1.63^{b}
15% Okara diet	55.50 ± 1.05^{b}	17.00 ± 2.83^{a}	8.00 ± 0.89^{ab}	153.00 ± 2.10^{b}

Mean value with the same alphabet as superscript within each column variable are non-significant ($p>0.05$).

h) Antioxidant Enzyme

Table 7 demonstrates the effect of 15% okara diet on selected organs of male Wistar rats. Following 43 days of experimentation, liver, kidney, and pancreas were harvested, homogenized, cold centrifuged, supernatants collected and used for determination of selected antioxidant enzyme levels. Interestingly, levels of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-s-transferase (GST), and glutathione peroxidase (GPX) for liver, kidney, and pancreas respectively were significantly decreased $p<0.05$ in the negative control group relative to the normal control. However, 15% okara diet supplemented fed group, and 6mg/kg glibenclamide treated group that was exposed to same levels of streptozotocin as the negative control showed a significant increase $p<0.05$ in the levels of selected antioxidant enzymes vis-à-vis negative control.

Table 7: Effect of 15% okara supplemented diet on Liver, Kidney and Pancreatic Antioxidant levels

	CATALASE	SOD	GSH	GST	GPX
GROUPS	LIVER				
Normal Control	39.60 ± 1.14^{c}	43.60 ± 2.30^{d}	83.20 ± 1.92^{d}	38.40 ± 1.14^{d}	33.00 ± 1.58^{c}
Negative Control	15.00 ± 1.58^{a}	22.00 ± 1.58^{a}	39.00 ± 1.58^{a}	11.20 ± 1.92^{a}	12.80 ± 1.48^{a}
Positive Control	26.20 ± 1.92^{b}	35.60 ± 1.14^{c}	62.00 ± 3.58^{c}	26.40 ± 2.30^{c}	25.00 ± 1.87^{b}
15% Okara Diet	24.20 ± 2.77^{b}	30.10 ± 1.14^{b}	56.40 ± 3.36^{b}	21.40 ± 2.30^{b}	22.20 ± 1.92^{b}
	KIDNEY				
Normal Control	33.60 ± 1.14^{d}	35.40 ± 2.30^{c}	64.80 ± 3.11^{d}	33.40 ± 2.70^{c}	32.20 ± 1.92^{c}
Negative Control	10.20 ± 1.92^{a}	16.80 ± 2.28^{a}	33.00 ± 3.16^{a}	18.20 ± 4.76^{a}	11.00 ± 1.87^{a}
Positive Control	25.20 ± 1.30^{c}	27.80 ± 1.64^{b}	47.20 ± 1.48^{c}	24.20 ± 0.84^{b}	24.60 ± 2.24^{b}
15% Okara Diet	21.00 ± 1.58^{b}	25.20 ± 2.39^{b}	42.00 ± 1.58^{b}	23.80 ± 2.25^{b}	23.40 ± 3.05^{b}
	PANCREAS				
Normal Control	30.20 ± 0.84^{c}	32.60 ± 1.14^{c}	54.40 ± 2.97^{c}	34.80 ± 1.30^{c}	35.00 ± 2.74^{c}
Negative Control	11.40 ± 1.14^{a}	12.60 ± 3.05^{a}	23.80 ± 2.39^{a}	12.80 ± 1.92^{a}	11.60 ± 1.82^{a}
Positive Control	23.80 ± 1.30^{b}	23.20 ± 1.79^{b}	45.20 ± 2.86^{b}	25.60 ± 2.30^{b}	23.20 ± 2.86^{b}
15% Okara Diet	23.40 ± 1.82^{b}	24.40 ± 2.41^{b}	43.00 ± 2.65^{b}	23.00 ± 2.55^{b}	22.60 ± 2.61^{b}

Means with the same alphabet as superscript within each column variable are non-significantly ($p>0.05$). Abbreviations denote SOD: Superoxide dismutase, GSH: reduced glutathione, GST: Glutathione-S-transfer, GPX: Glutathione peroxidase.

i) Histology of the pancreatic tissue

Plate I to III demonstrates the histological changes in Pancreatic, Liver, and Kidney tissues of four (4) groups at the end of the experimental period. (A) Section of the pancreas from normal control, with no visible lesions, observed. (B) Section of the pancreas from negative control, with a severe peritubular cellular infiltration. (C) Section of the pancreas from positive control, with no visible lesions, observed. (D) Section of the pancreas from 15% Okara diet, with no visible lesions, observed. (E) Section of the liver from normal control, with no visible lesions, observed. (F) Section of the liver from negative control, with random foci of single-cell hepatocellular necrosis. There is moderate Kupffer cell hyperplasia. (G) Section of the liver from positive control and (H) Section of the liver from 15% okara supplemented diet showed no visible lesions. Fig I to L section of the kidney showed no visible lesions for all groups.

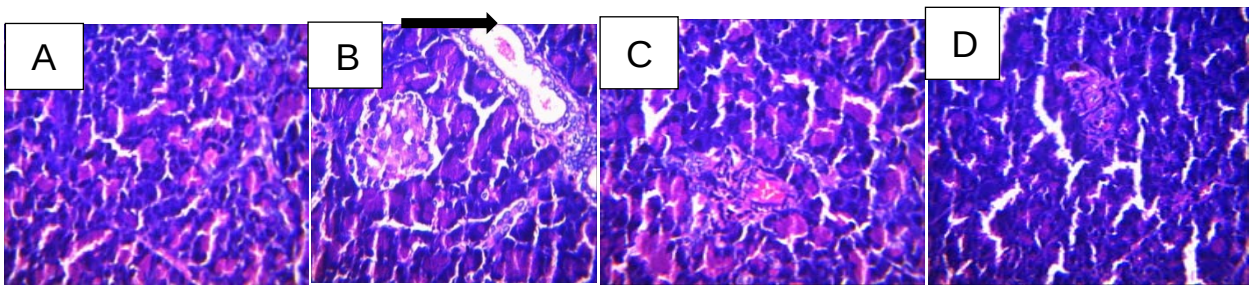


Plate I: Showing the histological section of Pancreatic Tissue

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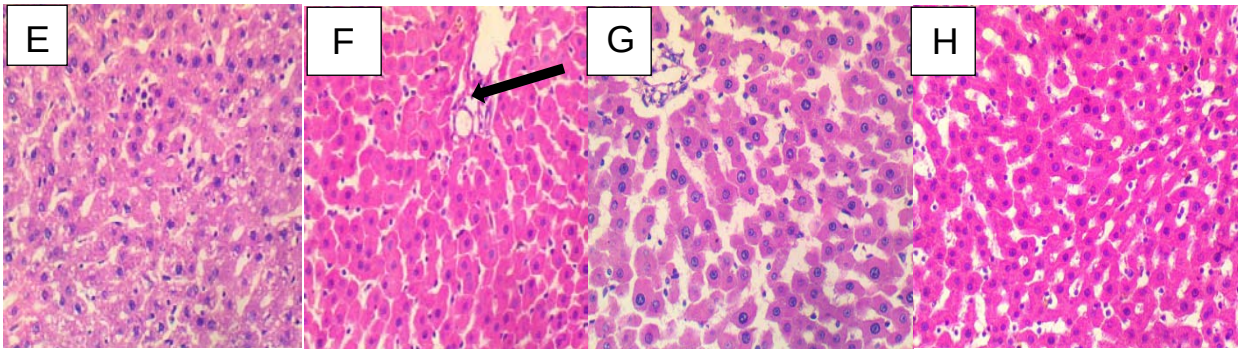


Plate II: Showing the histological section of Liver Tissue

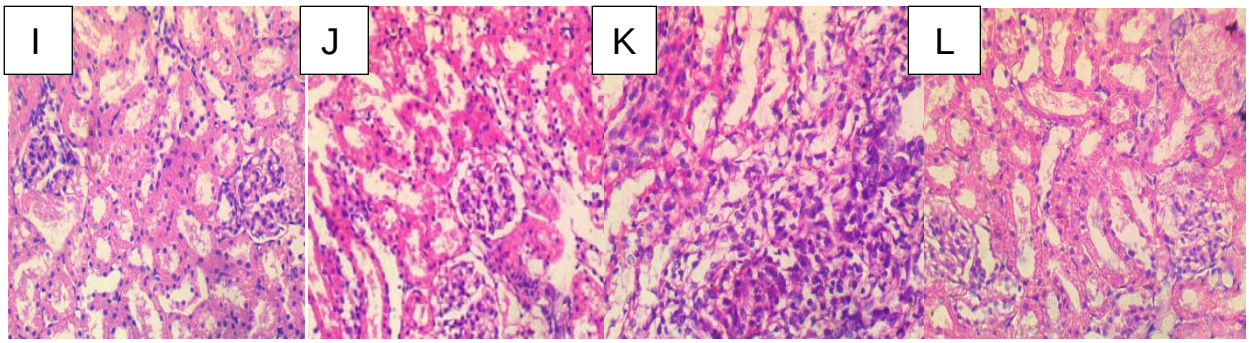


Plate III: Showing the histological section of Kidney Tissue

IV. Discussion

Diabetes mellitus is a serious health concern worldwide, and it is becoming the third most lethal disease of human and has continued to be on a rapid rise [24, 25]. In this light, it is imperative for an unrelenting research on evaluation and validation of nutraceutical potentials of plant food for the management of diabetes mellitus. Therefore, the present study sought to carefully examine the role of Okara supplemented diet on diabetic male Wistar rats.

Polyphagia is a condition characterized by an increased appetite leading to an increase in feed/food intake. It represents the initial signs of diabetes in humans as well as in experimental models [26, 7]. Owing to this finding, of course, our study demonstrated (table 2) that after diabetes induction with 50mg/kg streptozotocin from day 8 - day 15, the cumulative feed intake of the experimental groups were significantly increased $p<0.05$ vis-a-vis the normal control, and this may be as a result of the low levels of leptin; a critical signaling molecule in the hypothalamus influencing appetite and satiety [27]. Correspondingly, streptozotocin toxicity has been demonstrated to elicit a low level of leptin, and during uncontrolled Type 1 diabetes, plasma leptin levels rapidly fall whereas food intake increases [28,29]. However, 15% okara supplemented diets fed group from day 36 to day 43 showed no significant difference $p>0.05$ in cumulative feed intake relative to the normal control, and it may be attributed to the hypoglycemic potency of okara diet as seen in figure 2 and apparently, restoring the health status of the rats. Our result corroborates the finding of [7].

Streptozotocin-induced diabetes has been demonstrated to be associated with a severe reduction in body weight, perhaps, due to the degradation or loss of structural proteins that are clearly established to contribute to body weight gain [30]. Correspondingly, our study (figure 1) demonstrated that diabetes status was associated with a reduction in body weight as seen with the diabetic groups after an intraperitoneal injection of 50mg/kg streptozotocin. However, 15% okara supplemented diet feeding was significantly able to restore there rats body weight almost back to normal vis-à-vis negative control and this may be due to an improvement in diabetic status and other related abnormalities.

Diabetes mellitus is a metabolic disorder associated with an increased blood sugar levels, and of course, the result of our study figure 2, demonstrated a significant blood sugar elevation after intraperitoneal injection of 50mg/kg streptozotocin. However, 6mg/kg glibenclamide treatment and 15% okara diet supplemented diet significantly lowered the blood sugar levels, and the hypoglycemic ability of okara diet may be attributed to its high fiber content. High dietary fiber has been demonstrated to be beneficial, long lasting, and clinically relevant both in types 1 and type 2 diabetes [10] and this reports corroborate the findings of [31, 14].

To further confirm the hypoglycemic ability of okara diet, glucose-6-phosphate dehydrogenase, and glycated hemoglobin levels were evaluated (table 3).

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Glucose-6-phosphate dehydrogenase (G6PD), an enzyme that catalyzes the first step in the hexose monophosphate (HMP) shunt an alternative pathway for the catabolism of glucose to yield pentose sugar), and Glycated hemoglobin (HbA1c) formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma glucose levels [32]. As reported by [32], both markers are good predictors of diabetes. In this light, our study revealed that 15% okara diet supplemented diet restored the levels of G6PD and HbA1c vis-à-vis the negative control. Therefore, okara dietis a potent dietary agent for the management of diabetes.

Diabetic dyslipidemia shows high levels of plasma cholesterol (CHOL), triglyceride (TRIG), LDL-cholesterol (LDL), and low HDL-cholesterol (HDL) concentrations [33]. Similarly, alterations in lipid metabolism can cause lipotoxicity, which can further exacerbate diabetic complications [34]. Also, a high level of serum triglycerides, and a low level of High-Density Lipoprotein (HDL) are listed among the constellation for the medical conditions related to metabolic syndrome [35]. Of course, the result of the present study (table 4) revealed that the diabetic group (negative control) significantly $p<0.05$ had an elevated level of CHOL, TRIG, LDL and a reduced level of HDL relative to the normal control. Conversely, 15% okara supplemented diet feeding significantly normalized the lipid triad and hence may have also improved the diabetic condition of the rats. Additionally, our result corroborates the finding of [13, 14, 36].

Markers used to determine toxic effects of administered foreign substances to experimental animals are enzymes activities. Liver function enzyme ALP, is a membrane-bound enzyme meanwhile, ALT, and AST are cytosolic enzymes [37]. Therefore, high levels of ALP, ALT and AST respectively in the serum, are indicators of cell membrane permeability and a significant degree of damage to the liver [37]. Streptozotocin, a diabetogenic agent is associated with some degree of liver damage by several studies [38, 39]. In this light, of course, our result presented in Table 5 demonstrated that the levels of liver function biomarkers were significantly elevated in negative control which was exposed to 50mg/kg intraperitoneal injection of STZ relative to normal control. However, 15% okara diet supplementation potentiated a significant reduction in liver function biomarkers and this result suggests that some components of okara, namely; dietary fiber, dietary bioflavonoids, and isoflavones, could be associated with its ability in maintaining liver function.

Furthermore, one of the huge concerns of DM is its related complications, which can affect multiple vital organ systems [40, 41]. Creatinine, Urea, and electrolyte are selected indices of kidney function [42]. In this light, of course, the result of the present study table 6, underscored the favorable role of 15% okara diet supplementation in abrogating renal dysfunction induced by exposure to streptozotocin, as the was a significant reduction $p<0.05$ in markers of kidney function vis-à-vis the negative control.

Antioxidants are key players for the investigation of oxidant stress-related diabetic pathologies, and the activities of the antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase has been demonstrated to be reduced in diabetic conditions [43]. Correspondingly, DM is associated with an increased free radicals formation, and a decreased antioxidant capacity, consequently resulting to oxidative stress and a damage of cell components [44].

The result of the present study table 7, demonstrated that the negative control which remained untreated after 50mg/kg intraperitoneal injection of streptozotocin showed a significant decrease $p<0.05$ in levels of antioxidant enzymes CAT, SOD, GSH, GST, and GPX relative to the normal control for pancreas, liver and kidney respectively. The result is in tandem with the reports that kidney antioxidant enzyme activity declines in STZ induced animals as a consequence of the oxidative stress elicited by STZ [45]. Oxidative stress induced by streptozotocin (STZ) results to pancreatic beta cell damage [46], and a decline in the levels of liver antioxidant enzyme [47]. Interestingly, 15% okara supplemented diet caused a significant increase $p<0.05$ in antioxidant enzyme levels vis-à-vis negative control for all organs and this activity may be as a result of its composition which corresponds with the report of [48]; reported that, Okara contains phenolic compounds (106.7 mg gallic acid equivalents (GAE)/100 g) and flavonoids (32.7 mg quercetin equivalents/100 g) and showed antioxidant activity.

The histological observation of the pancreas figure A to D as contained in plate I for this study, revealed that the untreated group after 50mg/kg STZ injection (Negative control) had a severe a peritubular cellular infiltration. Perhaps, as a result of STZ diabetogenic action, by a direct irreversible damage to the pancreatic beta cells, this leads to degranulation, and loss of capacity to secrete insulin [49]. Correspondingly, the prevalence of diabetes mellitus is positively correlated with fatty infiltration of the pancreas [50]. However, the pancreatic tissue of the 15% okara supplemented diet fed group showed no visible lesions, and this highlights it ameliorative potency in the management of diabetes mellitus. The histological observation of the liver figure E to H, plate II shows that negative control (F) had random foci of single-cell hepatocellular necrosis, but this was reversed by 15% okara supplemented diet (H) as the was no visible lesion observed. However, the histological observation of the kidney figure I to L, plate III showed no visible lesion for all groups contrary the findings of [45] that histological observation of the kidney exposed to STZ was necrotic.

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V. Conclusion

A poor dietary habit has been demonstrated to be one of the key players in the development of diabetes mellitus, and a diet rich in dietary fiber has been highlighted to be a potent candidate for the management of DM. Thus, the result of our study shows that Okara diet; perhaps an excellent nutraceutical aids weight reduction, lower blood sugar and glycated hemoglobin levels and maintains a healthy level of lipid profile, liver, kidney, and antioxidant enzyme biomarkers. Conclusively, we recommend a supplementation of food with Okara.

Authors Contributions

Nwozo Sarah Onyenibe contributions to study were; conceptualized of the study, preparation of study design, supervision of the study, proofread and revised the study. Ikpeme Grace Edetcontributed to the study in the area of carry out experimentation, co-preparation of study design, collection of experimental data and reporting and revision of work. Nwawuba Stanley Udogadi contributed to the study by analysis and interpretation of data, preparation of manuscript and revision of the manuscript. All authors have approved the submitted version and agrees to be personally accountable for the authors contributions.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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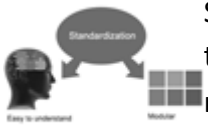


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Approach:

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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