RESEARCH ARTICLE

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Oral glucose administration impairs fertility parameters of male albino wistar rats

Oral glukoz uygulamasının erkek albino wistar ratlarının fertilite parametrelerine etkisi

Uduak Akpan OKON, Ndubuisi Emmanuel NWACHUKWU

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwalbom State, Nigeria

ABSTRACT

Introduction: Fertility issues has been one of the major concerns of health care givers and the patients; several factors can interfere with fertility parameters. This study was conducted to investigate the effect of glucose on male fertility parameters.

Materials and Methods: Eighteen male albino wistar rats weighing between 220 and 240 g were used. They were randomly assigned into three groups of six rats each. Group one served as control and was gavaged 5 mL of normal rat chow, groups two and three were gavaged 2.5 mg/kg and 5 mg/kg body weight glucose orally, twice daily as medium and high dose respectively twice daily for 21 days. On the 22nd day, the rats were sacrificed; blood samples were obtained by cardiac puncture; following standard procedure, the serum was obtained for hormonal (FSH, LH, Prolactin and Testosterone) assay using immunoassay kits. The testes were harvested and semen analysis was done.

Results: The results showed that FSH levels in medium and high dose groups were significantly ($p< 0.01$) higher. There was a significant ($p< 0.01$) dose-dependent decrease in LH levels. A significant ($p< 0.01$) dose-dependent decrease was observed in sperm motility. Also, a significant ($p< 0.01$) decrease was observed in percentage sperm concentration and percentage normal sperm morphology.

Conclusion: We therefore infer that excess glucose intake adversely affects male fertility parameters in male albino wistar rats.

Keywords: *Fertility, glucose, sex hormones, male, semen analysis*

ÖZ

Giriş: Fertilite sorunları, hem sağlıkçıların hem de hastaların başlıca sorunu haline gelmiştir. Fertilite parametreleri çeşitli faktörlerden etkilenmektedir. Bu çalışma, glukozun erkek fertilite parametreleri üzerindeki etkisini araştırmak için yürütülmüştür.

Materyal ve Metod: Bu çalışmada, ağırlıkları 220-240 g arasında değişen 18 erkek albino wistar rat kullanıldı. Ratlar, her bir grupta altı rat olacak şekilde rastgele üç gruba ayrıldılar. Birinci grup kontrol grubu olarak seçildi ve 5 mL normal salin solüsyonu ile beslendiler. İkinci ve üçüncü gruplar ise vücut ağırlığına bağlı olarak sırasıyla orta ve yüksek doz olarak 2.5 mg/kg ve 5 mg/kg ile 21 gün boyunca günde iki kez beslendiler. Yirmi ikinci günde, ratlar öldürüldü ve kardiyopünktür ile kan örnekleri alındı. Standart prosedür takip edildi ve seruma immünoserolojik kit kullanılarak hormonal (FSH, LH, prolaktin, testosteron) analiz yapıldı. Testisler alındı ve sperm analizi yapıldı.

Bulgular: Orta ve yüksek doz gruplarındakı FHS düzeyi anlamlı derecede yüksekti ($p< 0.01$). LH düzeylerinde doza bağlı düşüş gözlemlendi. Anlamlı ($p< 0.01$) bir doza bağlı düşüş, sperm motilitesinde izlendi. Ayrıca, anlamlı ($p< 0.01$) bir düşüş, sperm konsantrasyonu yüzdesi ve normal sperm morfoloji yüzdesinde görüldü.

Sonuç: Dolayısıyla, aşırı glukoz alımının erkek albino wistar ratlarda erkek fertilite parametrelerini olumsuz olarak etkilediği sonucuna varıyoruz.

Anahtar Kelimeler: *Fertilite, glukoz, cinsiyet hormonları, sperm analizi*

Yazışma adresi/Correspondence: Uduak Akpan OKON

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Uyo University, Uyo, Akwalbom State, Nigeria

Telefon/Tel: *+23 480 23147921* • ***E-posta/E-mail:*** *chairmo2013@gmail.com*

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INTRODUCTION

The ability to reproduce is one of life's essential functions and as such factors that affect this ability are of vital importance to mankind. Fertility issues had been one of the major concern of health care givers and the likes. Studies have shown that more than 30% of fertility problem are due to male factor (1). Several factors can interfere with fertility parameters, factors such as drug treatment; environmental toxins, air pollution and stress have harmful effects on fertility. Various investigative parameters of fertility include semen analysis (sperm motility, count, morphology volume, fracture level and pH) and hormonal assay (2,3).

Male fertility depends on the quality of the semen, the two most important factors being the number or count and motility of the spermatozoa. Decreased sperm production (Oligospermia) is a common cause of infertility in the male and a count of less than 15 million spermatozoa is considered incompatible with fertility (2). The accuracy of the analysis of semen can be affected by different factors which may change with respect to time (4). Therefore, a future test is needed for confirmation of fertility (5).

Normal levels of Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH) are needed for spermatogenesis to take place. FSH help induce the conversion of primary spermatocytes to secondary spermatocytes. FSH promotes the synthesis of androgen-binding protein by the Sertoli cells which attach to FSH receptors on their basolateral membranes which is necessary for spermatogenesis to occur (6). LH induces the secretion of testosterone from the Leydig cells which promotes spermatogenesis (7). Higher FSH concentration is usually considered to be a reliable indicator of germinal epithelial damage and also associated with azoopermia and severe oligospermia (9). This factor is the probable cause of the significant decrease in sperm concentration due to increased destruction of germ cells that give rise to sperm. Decreased level of testosterone may contribute to the decrease in normal sperm morphology since testosterone is required for the completion of meiotic division during spermatogenesis and for the early stages of spermatid maturation (10).

Physiologic levels of prolactin in males enhance luteinizing hormone-receptors in Leydig cells, resulting in testosterone secretion, which leads to spermatogenesis; it potentiates actions of testosterone in the male (11-13). Highly elevated levels of prolactin (Hyperprolactinaemia) decrease the levels of sex hormones-estrogen in women and testosterone in men, it can cause impotence or reduced libido in men suggesting its definite role in male infertility (13-16). Hyperprolactinaemia may also be the result of disease of other organs such as the liver, kidneys, ovaries and thyroid (17).

Glucose is an important source of energy in the body. Even though man consumes almost as much energy from carbohydrate as from fat, the body prefers to oxidize carbohydrate and store the fat; the carbohydrate stores of the body are limited (18,19). Glucose metabolism via glycolysis is the principal route for the production of ATP molecules. Substantial evidence exists that sperm ATP production via glycolysis is required for mammalian sperm function and male fertility. Inhibition of glycolysis impairs motility (20). Glycolysis is enzyme - regulated, as such factors that inhibit the regulating enzymes slow down glycolysis. Phosphofructokinase-1 is the key regulating enzymes of glycolysis and is inhibited by citrate and cAMP when high levels of glucose are present, which signals high energy state, this slows down glycolysis (21). Also, the seminal vesicles can isomerize glucose to fructose to meet the need for this particular monosaccharide (22).

Sperm concentration, motility and viability are crucial indices that determine the potential of sperm to fertilize an ovum. Poor semen characteristic has been linked to damage spermatocyte DNA (23,24). It has also been reported that the integrity of spermatocyte DNA is protected by zinc and citric acid secreted from the prostate gland (25). Other studies have revealed that excessive consumption of carbohydrate (glucose) based diet contributes to obesity which in turn impedes male fertility. Therefore, the role of nutrition vis-a-vis glucose and hormonal factors in male fertility is very important. This research therefore aims to investigate how high or low glucose intake will affect sperm qualitative and quantitative characteristic and the relevant fertility hormones.

MATERIALS and METHODS

Animal Preparation, Experimental Groupings and Treatment

In this randomized study, eighteen adult male albino wistar rats were used and randomly assigned one of three groups such that each group had six animals (6). Group 1 served as the control group fed with normal rat chow (feed). Group 2 served as the medium dose group and were given 2.5 mg/kg glucose orally, twice daily. Group 3 served as the high dose group and received 5 mg/kg glucose orally, twice daily. All animals had access to water ad libitum. The cages were well ventilated, exposed to normal temperature and 12/12 hours light/dark cycle. After fourteen days of acclimatization, oral administration of glucose to groups 2 and 3 commenced.

The animals were sacrificed after 21 days. All experiments were examined and approved by the appropriate ethics committee of our University and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Sperm Cell Concentration

Testes was crushed into pieces, diluted in 5 mls of normal saline and allowed for 5-10 minutes to enable the spermatozoa to spread out into the diluents solution. 1 mL of supernatant was diluted in 100 mls solution e.g 0.1 of solution 1 + 5 mls of solution. 0.01 of the suspension was loaded into a charged Neubauer counting chamber and cover slipped. It was allowed to rest for 10 minutes and observed microscopically. Number of cells was counted in millions/mL (26-28).

Sperm Morphology

1 mL of seminal fluid was diluted with 20 mls of buffered formol saline and then 0.01 mL of the solution was loaded on a grease free slide with cover slip and viewed under a microscope and the following were observed: tail defect, neck defect, mid-piece defect, head defect and percentage of normal morphology was determined (26-28).

Sperm Motility

1 mL of seminal fluid was diluted with 20 mls of buffered formol saline and 0.01 mL of the solution was loaded on a grease free slide and covered with a cover slip and observed microscopically(26-28).

Serum FSH, LH and Prolactin Measurements

The FSH, LH and prolactin were determined based on the principle of sandwich method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact FSH, LH and prolactin molecule. The test sample is allowed to react simultaneously with two antibiodies, resulting in the FSH, LH and prolactin molecules being sandwiched between the solid phase and enzyme - linked antibodies.

After incubation, the wells were washed with washing solution to remove unburned labeled antibodies. Tetra methyl benzidine substrate was added and incubated, resulting in the development f a blue colour. The colour development is stopped with addition of stopping reagent, changing the colour to yellow. The concentration of FSH, LH and prolactin was directly proportional to the colour intensity of the test sample (26-28).

Serum Testosterone Measurement

Testosterone level was determined using competitive microplate enzyme immunoassay. Plates are coated with anti-testosterone antibodies. Calibrator specimen was first added to microplate well. Enzyme testosterone conjugate was added. Testosterone present in the sample competes with enzyme-testosterone conjugate for building with anti-testosterone counted microplate to form an antigen-antibody complex. Unbound conjugate was removed by washing. The enzyme activity in the antibody-bound fraction was inversely proportional to the native testosterone concentration. The enzyme activity was revealed by colour change in tetramrthylbenzidine substrate solution (26-28).

Statistical Analysis

All results were presented as mean + standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the least significant difference (LSD) procedure for significant F values, (p= 0.05) was considered significant. Computer software SPSS and Excel Analyzer was used for the analysis.

RESULTS

Sperm Cell Concentration

The percentage sperm concentration in the control, medium dose and high dose groups were 69.67 + 2.08%, 53.67 + 2.74% and 41.33 + 0.21% respectively. There was a significant decrease ($p< 0.001$) in percentage sperm concentration in both medium and high dose groups compared to control.There was also a significant ($p< 0.01$) dose - dependent decrease in percentage sperm concentration in the high dose group when compared to the medium dose group (Figure 1).

Sperm Morphology

The mean values of percentage normal sperm morphology in the different groups were 81.33 + 0.56%,

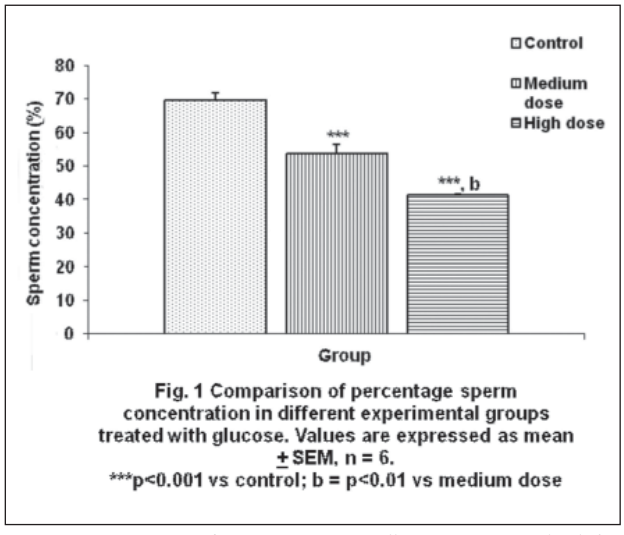

Fig. 1 Comparison of percentage sperm concentration in different experimental groups treated with glucose. Values are expressed as mean ± SEM, n = 6.
***p<0.001 vs control; b = p<0.01 vs medium dose

Figure 1. *Comparison of percentage sperm cell concentrationin the different groups.*

54.00 + 1.10% and 58.00 + 0.37% respectively. A statistically significant (p< 0.001) decrease in percentage normal sperm morphology was observed in both medium and high dose groups when compared to the control group. A significant increase (p< 0.01) in percentage normal sperm morphology was observed in high dose group compared to medium dose group. (Figure 2).

Sperm Motility

Mean values of fast progressive movements (motility) of sperm in control, medium dose and high dose groups were 71.67 + 2.79%, 56.67 + 2.79% and 56.67 + 2.11% respectively. There was a significant (p< 0.01) decrease in fast progressive movement of sperm in both medium and high dose when compared to the control (Figure 3).

Comparison of FSH Levels in the Different Groups

The level of FSH for the control, medium dose and high dose groups were 0.10 + 0.00 mm/mL, 0.17 + 0.02 mm/mL and 1.27 + 0.28 mm/mL respectively. The FSH levels in the medium dose and high dose groups were significantly higher (p< 0.01) than the control. There was significant (p< 0.01) dose - dependent increase in FSH level in the high dose group compared to the medium dose group (Figure 4).

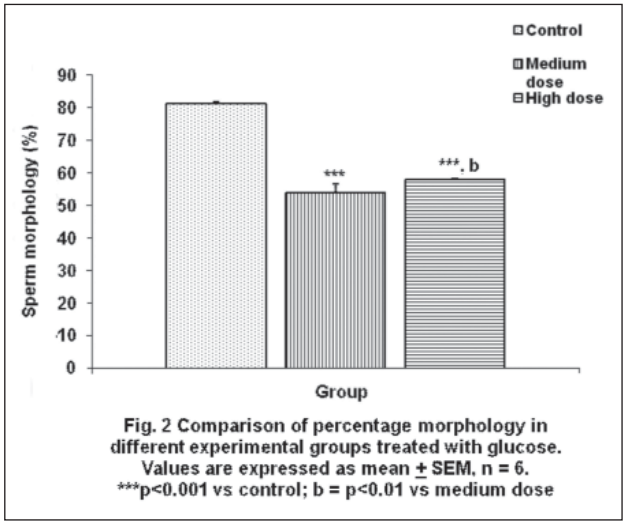

Fig. 2 Comparison of percentage morphology in different experimental groups treated with glucose. Values are expressed as mean ± SEM, n = 6.
***p<0.001 vs control; b = p<0.01 vs medium dose

Figure 2. Comparison of percentage sperm morphology in the different groups.

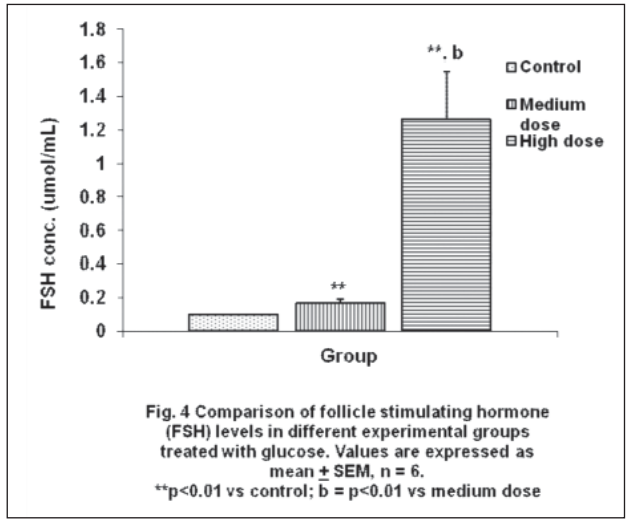

Fig. 4 Comparison of follicle stimulating hormone (FSH) levels in different experimental groups treated with glucose. Values are expressed as mean ± SEM, n = 6.
**p<0.01 vs control; b = p<0.01 vs medium dose

Figure 4. Comparison of FSH levels in the different groups.

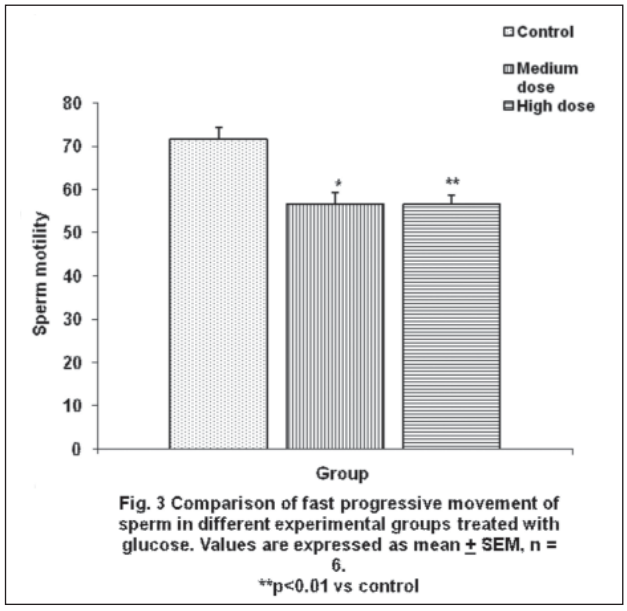

Fig. 3 Comparison of fast progressive movement of sperm in different experimental groups treated with glucose. Values are expressed as mean ± SEM, n = 6.
**p<0.01 vs control

Figure 3. Comparison of percentage sperm motility in the different groups.

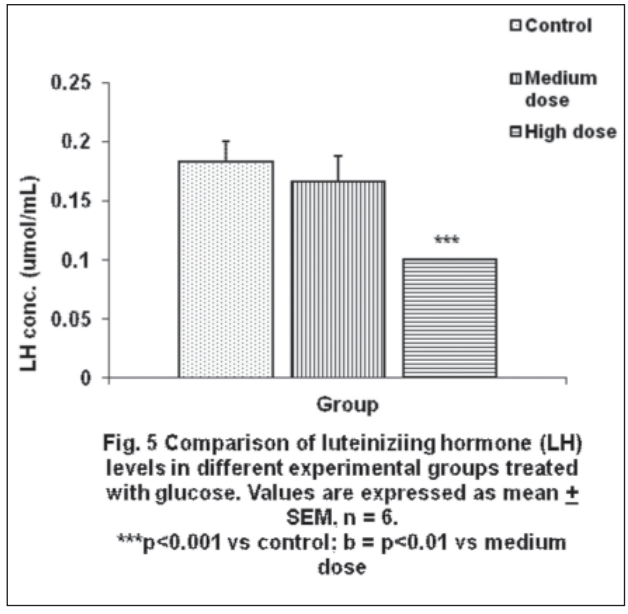

Fig. 5 Comparison of luteiniziing hormone (LH) levels in different experimental groups treated with glucose. Values are expressed as mean ± SEM, n = 6.
***p<0.001 vs control; b = p<0.01 vs medium dose

Figure 5. Comparison of LH levels in the different groups.

Comparison of LH Levels in the Different Groups

Mean values of LH levels in the control, medium dose and high dose groups were 0.18 + 0.02 mm/mL, 0.17 + 0.02 mm/mL and 0.10 + 0.00 respectively.

LH level in high dose group was significantly lower ($p< 0.01$) than the control. There was significant ($p< 0.01$) dose - dependent decrease in the high dose group compared to the medium dose group (Figure 5).

Comparison of Prolactin Levels in the Different Groups

Mean values of prolactin level in control, medium dose and high dose groups were 0.12 + 0.02 mm/mL, 0.12 + 0.02 mm/mL and 0.01 + 0.00 mm/mL respectively. There was no statistically significant difference in prolactin levels in the different experimental groups (Figure 6).

Comparison of Testosterone Levels in the Different Groups

Mean values of testosterone level in control, medium dose and high dose groups were 8.12 + 2.01 mm/mL, 13.25 + 3.00 mm/mL and 9.43 + 1.40 mm/mL respectively. There was no statistically significant difference in testosterone in the different groups (Figure 7).

DISCUSSION

This work investigated the effect of oral glucose administration on fertility parameters of male albino rats with some exceptional findings on some aspect of the result. The results obtained from the study reveal some interesting effects on male fertility. The contrasting result on the gonadotropins - FSH, and LH strongly suggests that a parallel change in the secretion of these hormones is not always the case. While FSH level showed significant dose-dependent elevation, LH level decreased significantly in a dose dependent pattern.

The pulsatile secretion of GnRH is regulated by negative feedback signals of inhibin for FSH specifically and testosterone for LH respectively. Increase glucose blood concentration or products of its metabolism therefore appears to selectively interfere with the inhibin negative feedback pathway while sparing the testosterone pathway. Hence the significant elevation of FSH level as seen in the result. LH is the primary stimulus for the secretion of testosterone (29). The decreased level of LH in the result might have contributed to the marginal reduction of testosterone levels since the stimulus for the secretion was deficient. It is possible that with longer duration of glucose administration, this effect might be more pronounced.

There was no significant change in prolactin level. It is known that physiologic levels of prolactin enhance luteinizing hormone receptors in Leydig cells, resulting in testosterone secretion, which promote spermatogenesis (11). Prolactin therefore has an indirect role in promoting spermatogenesis. The moderate decrease of prolactin concentration in this study could have contributed to the decrease in sperm concentration. The complimentary role of prolactin probably having been abolished.

Physiologic levels of FSH and LH enhance spermatogenesis. FSH is involved in the proliferative stage of spermatogenesis as well as stimulates Sertoli cells to secrete aromatase which converts testosterone to estrogen, which is very important for spermiogenesis. There was a

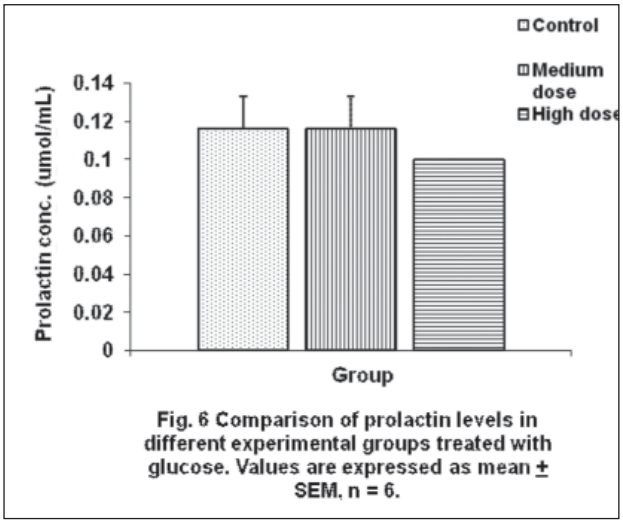

Figure 6. *Comparison of prolactin levels in the different groups.*

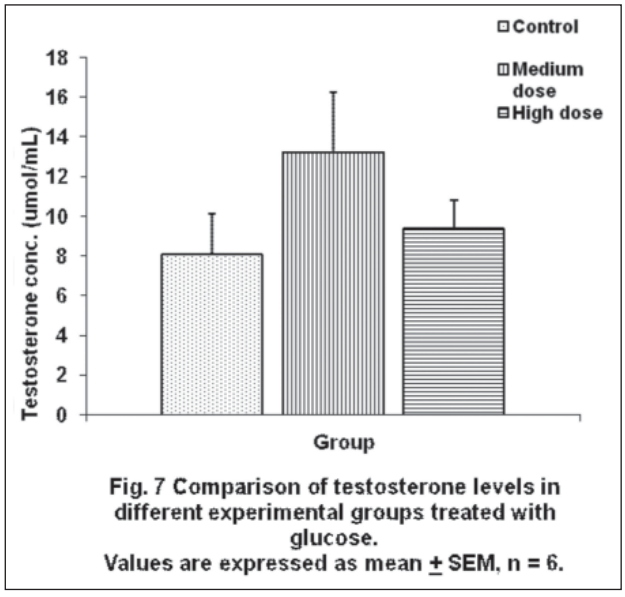

Figure 7. *Comparison of testosterone levels in the different groups.*

significant dose - dependent elevation of FSH level in the result. Higher FSH concentration is usually considered to be a reliable indicator of germinal epithelial damage and also associated with azoopermia and severe oligospermia (9). This factor may probably account for the significant decrease in sperm concentration due to increased destruction of germ cells that give rise to sperm, by some unknown spermatozoa targeted toxic metabolite of glucose. A normal level of testosterone is required for the completion of meiotic division during spermatogenesis and for the early stages of spermatid maturation (10). Though, the reduction in testosterone levels was marginal, it is likely that other factors not yet understood could escalate the effect of this marginal decrease which in turn impair normal sperm maturation process with subsequent decrease in normal sperm morphology.

The metabolism of glucose through glycolysis is the mainway for ATP synthesis. Many researchers have shown that sperm ATP production through glycolysis is needed for mammalian sperm function and male fertility; hinderance of glycolysis negatively affect motility (20). Glycolysis is enzyme - regulated, as such factors that inhibit the regulating enzymes slow down glycolysis. When high levels of glucose are present, as could have occurred for the duration of the glucose administration, the enzyme phosphofructokinase-1 might have been inhibited. Phosphofructokinase-1 a key regulating enzymes of glycolysis is inhibited by citrate and cAMP when high levels of glucose are present, which signals high energy state slows down glycolysis (21). The significant decrease in fast progressive movement of sperm (motility) observed in the result could therefore be due to the inhibition of the enzyme phosphofructokinase - 1 which led to inhibition of glycolysis with consequential impairment of sperm motility.

CONCLUSION

As evident from our result, oral glucose administration imposes a dose-dependent adverse effect on fertility facilitators and indices in male albino wistar rats. We therefore infer that excess glucose intake adversely affects male fertility parameters in male albino wistar rats.

Ethical Approval

All authors hereby declare that all experiments have been examined and approved by the ethical committee of the University of Uyo on Animal Research and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CONFLICT of INTEREST

All authors have declared no conflict of interest.

ACKNOWLEDGMENTS

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