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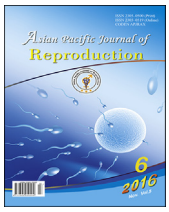

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Studies on the protamine 1 gene in the sperm DNA of male albino rats treated with local gin using *Bse*R I endonuclease

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ABSTRACT

Objective: To investigate the impact of local brewed gin (ogogoro) on the integrity of sperm DNA through the evaluation of the protamine 1 (*PRM1*) gene.

Methods: Twenty four 9-week-old male albino rats were divided into five groups; the first group which served as the control were fed with normal fish feed and water *ad libitum* while the rest were fed with different volumes of the local gin (1, 2, 3 and 4 mL respectively) in conjunction with the normal fish feed and water for 24 d. The caudal epididymis was macerated in saline water and the sperm solution collected in an Eppendorf tube. The extraction of DNA was done using Jena Bioscience DNA preparation kit and the protocol was based on the spin column based genomic DNA purification from blood, animal and plant cells. The primer was designed to detect mutation in Protamine 1 gene associated with male infertility. *Bse*R I, a restriction endonuclease was used to digest the amplicon at 37 °C for 10 min and then inactivated at 80 °C for 20 min. The digest was separated on 2% agarose gel.

Results: There was variation in the thickness of bands and the thinnest band was observed in the group that consumed the highest volume of local gin. There was also a close similarity between the amplicons from the group fed with 1 mL of ogogoro and the control group that were given distilled water. There was no visible digestion by *Bse*R I. There was a significant increase in the body weight across the entire experimental group and the highest weight was recorded in the 4 mL group. Multiple tumors were also seen in the livers of all the experimental groups.

Conclusion: This study has shown that local gin can affect the sperm DNA by making it susceptible to damage because of the reduced viability of the *PRM1* gene whose product i.e., PRM1 protein is needed for the proper compaction of the sperm DNA.

1. Introduction

Local gin is a clear, colorless, mobile, volatile, alcoholic drink that is brewed locally in western Africa. It is commonly called “Sapele water”, “Ogogoro”, “Paraga”, “Kaikai”, “Etonto”, “wuru”, “robirobi” in Nigeria though other countries have their own home brew such as “umkomboti” in South Africa, Ghana's “Nsafufuo” or “muratina” and “chang'aa” in Kenya.

In Africa, alcoholic beverages were consumed for pleasure after brewing or tapping [1] and they were rarely traded in the market. This drink is of historical significance in Ghana and Nigeria because, as a local gin, colonial administrators barred it in an attempt to control the West African liquor trade in the early part of the last century [2].

Nigeria is one of the world's leading consumers of alcoholic beverages with an average of about 15 L of pure alcohol per capita per annum [3]. It is an essential part of numerous religious and social ceremonies thus it carries a substantial cultural and economic significance in Nigeria. It is also used during traditional ceremonies such as pouring libation, weddings and funerals.

Little attention is placed on male infertility in developing countries because of the widely erroneous belief that infertility is a female problem [4]. The male contribution to infertility among couples worldwide has been estimated to be about 33% and in Nigeria, the male partners' contribution to sub fertility is estimated to be about 54% based on semen analysis alone [5].

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Moreover, approximately 15% of patients with male factor infertility have a normal semen analysis [6] whereas in 8% of men with normal sperm parameters, different forms of sperm DNA damage are found [7].

Testicular germ cells are highly susceptible to damage from a number of toxic chemicals in comparison to somatic cells because they have higher amount of polyunsaturated fatty acids that are vulnerable to oxidation by free radicals arising from phagocytic Sertoli cells. These reproductive toxins are encountered in many ways; industrially and environmentally, therapeutically and self-administered as recreational drugs [8]. Studies have reported increased levels of gonadotropins and a decreased level of testosterone in infertile males when compared with the fertile ones [9]. Many studies have been undertaken to determine the temporal relationship between alcohol and fertility. In human, high alcohol consumption is associated with serious disorders of spermatogenesis. Among chronic alcoholics, there is impairment of spermatogenesis and reductions in sperm counts and testosterone levels [10].

Spermatogenesis is a tightly regulated and complex biological process of cellular differentiation that results in the production of haploid male germ cells. During spermatogenesis, a complex and dynamic process of proliferation and differentiation occur as spermatogonia are transformed into mature spermatozoa. This unique process involves a series of meioses and mitoses, changes in cytoplasmic architecture, replacement of somatic cell-like histones with transition proteins, and the final addition of protamines, leading to a highly packaged chromatin.

The presence of DNA damage in the male germ line has been linked with a variety of adverse outcomes such as low fertilization rates, decrease in embryo implantation, miscarriage, cancer and other diseases in the offspring [11]. It is also known that some paternal genes are crucial for early embryo development. Most genes in spermatozoa are hypermethylated and those regions hypomethylated are usually related to development regulators, biosynthetic or metabolism loci [12]. The organization of sperm nuclear DNA takes place in the haploid stage of spermatogenes is called spermiogenesis. In the testicular phase, histones are replaced at first by lysine-rich transition proteins (TPs) and then by protamines. Two theories have been proposed to explain the phenomenon of why ejaculated human spermatozoa possess anomalies in their nuclear DNA. The first theory arises from studies performed in animal models and is linked to the unique manner in which mammalian sperm chromatin is packaged. Endogenous nicks in DNA have been shown to be normally present at specific stages of spermiogenesis in rats and mice [13].

The second theory of why DNA damage is present in ejaculated human spermatozoa arises from the use of the TUNEL assay as a marker of apoptosis. A number of studies have therefore stated that the presence of TUNEL-positive sperm indicates that these sperm are indeed apoptotic [14].

This study was designed to investigate the implication of locally brewed drink (ogogoro) on the sperm nuclear DNA integrity analyzed through molecular technique of polymerase chain reaction (PCR). This is due to the fact that some tests such as semen analysis, sperm chromatin dispersion (SCD) test, The terminal deoxynucleotidyl transferase-mediated (TdT) deoxyuridine triphosphate (dUTP) nick end labeling assay (TUNEL) are limited to sperm counts and fragmentation analysis which may not be informative enough about the integrity of the sperm DNA as researchers have discovered that even in the absence of fragmentation, some damage may be detected in some genomic regions [15].

In the human, it has been known for many years that the chromatin of the mature sperm nucleus can be abnormally packaged. In addition, abnormal chromatin packaging and nuclear DNA damage appear to be linked [16]. There is also a strong association between the presence of nuclear DNA damage in the mature spermatozoa of men and poor semen parameters [17].

The aim of the research was to investigate the impact of local brewed gin (ogogoro) on the quality and integrity of sperm DNA.

2. Materials and methods

2.1. Animal and experimental design

Twenty-four 9-week-old albino rats with an average weight of 166.6 g were kept in clean cages and acclimatized for 1 week. Animals were randomly divided into five groups; the first group which served as the control were fed with normal fish feed and water *ad libitum* while the rest were fed with different volumes of the local gin (1, 2, 3 and 4 mL respectively) in conjunction with the normal fish feed and water for 18 d. Experiment was carried out in the animal house of the University of Lagos, Lagos, Nigeria in accordance with the rules in Nigeria governing the use of laboratory animals as acceptable internationally [18]. During the induction period, eight rats died. The feeding was halted for 2 weeks and the animals were finally fed for 6 d with the gin and sacrificed.

2.2. Epididymal sperm preparation

The caudal epididymis was macerated in saline water and the sperm solution collected in an Eppendorf tube. The molecular analysis was carried out at the molecular laboratory of FowM biotechnology company, Jibowu, Lagos state.

2.3. Sperm DNA extraction

The extraction of DNA was done using Jena Bioscience DNA preparation kit and the protocol was based on the spin column based genomic DNA purification from blood, animal and plant cells. Prior to the extraction, 500 μL dd-water was added to the proteinase K tube, 150 μL of dd-water to RNase A tube and 48 mL 96%–99% ethanol to the washing buffer bottle.

The sperm solution was centrifuged at 10 000 r/min for 1 min and the supernatant was discarded. 300 μL lysis buffer was added and 2 μL RNase A to the cell pellet which was then vortexed for 30 s. 8 μL proteinase K was added and incubated for 10 min at 60 °C. It was then cooled for 5 min. 300 μL binding buffer was added and vortexed after which the tube was placed on ice for 5 min and then centrifuged for 5 min at 10 000 r/min.

Spin column was placed into a 2 mL collection tube and 100 μL of activation buffer was added into the spin column. This was centrifuged at 10 000 r/min for 30 s and the flow-through was discarded. Supernatant was transferred to this spin column and centrifuged for 1 min at 10 000 r/min. Flow-through was discarded.