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Evaluation of the Effect of High Dose Kola Nut Ethanolic Extract on Testicular Enzymes in Male Albino Rats

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ABSTRACT

Original Research Article

The present study investigated the effect of high dose of kola nut (Cola nitida) ethanolic extract on the testicular enzyme activities of adult male albino Wistar rats. Twelve adult male albino Wistar rats were randomly divided into three groups of four rats each. Group 1 acted as control and was given only normal rat meal and distilled water. Groups 2 and 3 were given kola nut ethanolic extract at a level of 100 mg/kg body weight and 400 mg/kg body weight correspondingly. The medication was given for a period of four weeks. At the end of the experimental period, rats were slaughtered and testicular tissues were collected for biochemical investigation. Spectrophotometric determination of acid phosphatase (ACP) and lactate dehydrogenase (LDH) activity in the testis. Data were analysed using SPSS version 27.0 and differences between groups were compared using one-way analysis of variance (ANOVA) with Tukey's post hoc test. The average ACP and LDH activity of the control group were 227.00 ± 3.37 U/L and 13.25 ± 1.71 U/L respectively. The recorded ACP and LDH activities for the low-dose group were 230.50 ± 2.65 U/L and 17.75 ± 1.26 U/L respectively. The recorded ACP and LDH activities for the high-dose group were 233.33 ± 3.11 U/L and 25.50 ± 2.89 U/L respectively. Significant variation of ACP activity was detected across three groups ($F = 4.536$, $P = 0.043$). Similarly, the LDH activity was considerably different among groups ($F = 35.903$, $P < 0.001$). The gradual increases in the activities of ACP and LDH, especially in high dose group, indicate that administration of kola nut ethanolic extract at high doses can bring about changes in the testicular enzyme activity and cellular metabolism. These findings may suggest potential implications of overexposure to kola nut ethanolic extract on testicular function and the need for caution with long term or high dose use.

Keywords: Kola nut, Cola nitida, ethanolic extract, testis, acid phosphatase, lactate dehydrogenase, albino Wistar rats, reproductive damage.

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INTRODUCTION

Kola nut is a traditional and cultural plant originating from the seeds of Cola nitida and Cola acuminata. It is commonly consumed in various parts of West Africa, notably Nigeria. Its consumption is strongly embedded in social, cultural, ceremonial and traditional activities where the nut is often given to visitors, used in traditional gatherings and eaten for its alleged stimulating effects. Kola nut is also used traditionally for energy, to alleviate fatigue, hunger and physical depletion. The increasing popularity of Kola nut intake has stimulated scientific interest in its nutritional, pharmacological and toxicological qualities especially when taken in large numbers or at high doses. The biological benefits of kola nut are mostly due to its various phytochemical ingredients such as caffeine, theobromine, tannins, flavonoids, alkaloids and other phenolic chemicals [1,2]. The Kola nut is a member of the genus Cola, family Malvaceae, mainly Cola nitida and Cola acuminata. Cola acuminata is a tropical evergreen tree, reaching substantial heights, that bears unique follicles, each bearing numerous seeds. The seeds, generally known as kola nuts, may be reddish or whitish in colour, depending on the species and variation. The nut has a particular bitter flavour and contains biologically active chemicals responsible for its typical stimulating effects [3,4]. Among these ingredients caffeine is of special importance due to its capacity to stimulate the central nervous system, improve alertness and influence various physiological processes. Kola nut has been reported to contain about 2-4% caffeine, varied levels of theobromine, tannins, saponins, flavonoids and other secondary metabolites [5]. Moderate intake may create favourable stimulant effects, but higher intake may expose the user to high quantities of caffeine and other bioactive chemicals that may have detrimental physiological and biochemical effects. The reproductive system is extremely vulnerable to environmental, dietary, pharmacologic and toxicologic influences. In males, the testes have two major functions: generation of spermatozoa by spermatogenesis and synthesis of steroid hormones, namely testosterone. These tasks need

coordinated interaction between Leydig cells, Sertoli cells, germ cells, seminiferous tubules and the hypothalamic-pituitary-gonadal axis. Maintenance of normal testicular structure and function also requires optimal energy metabolism, membrane integrity, antioxidant protection and appropriate hormonal regulation. Therefore, exposure to drugs that can modulate oxidative balance, cellular metabolism or endocrine activity may have negative effects on testicular function and male fertility [6]. Kola nut contains caffeine and other biologically active chemicals which may affect reproductive function if ingested in excess. Caffeine may impact intracellular signalling, cellular metabolism and hormonal regulation. However, prolonged exposure to stimulant substances could lead to increased metabolic activity and may contribute to oxidative stress. The testes are especially susceptible to oxidative damage, since the membranes of sperm cells contain polyunsaturated fatty acids that are susceptible to lipid peroxidation. Excessive reactive oxygen species generation may potentially disrupt germ-cell development, change Sertoli-cell activity, damage cellular membranes and compromise spermatogenesis. The reproductive effects of caffeine and other phytochemicals may be dose-dependent, duration of exposure and individual susceptibility, however examination of high-dose kola nut exposure is relevant as consumption habits may involve recurrent or excessive intake [7]. Testicular enzymes are important biochemical markers in assessing cell activity and probable tissue damage. Acid phosphatase (ACP) is a lysosomal enzyme involved in the hydrolysis of phosphate-containing substrates and is linked with several cellular and metabolic processes. Testicular acid phosphatase activity in the male reproductive system has been correlated with the functional status of testicular cells and processes related to spermatogenesis. Thus, variations in ACP activity may be reflecting changes in cellular integrity, lysosomal activity or metabolic function inside the testes. Lactate dehydrogenase (LDH), in contrast, is an essential enzyme in anaerobic glycolysis that catalyses the reversible conversion of pyruvate to lactate while facilitating the regeneration of NAD+. The glycolytic pathways and LDH activity are essential for testicular energy metabolism because growing germ cells require a large amount of energy during spermatogenesis [8]. Thus, variations in testicular LDH activity may indicate variations in energy metabolism and cellular function. An increase or reduction in enzyme activity after exposure to a chemical or plant extract may suggest adaptive metabolic responses, cellular stress or tissue damage depending on the type and extent of change. Therefore, the biochemical assessment of ACP and LDH provides a very easy method to the investigation of putative effects of kola nut exposure on testicular tissue.It has been shown in previous experimental research that exposure to high quantities of physiologically active plant compounds may affect reproductive hormones, testicular enzymes, oxidative state and sperm characteristics. A number of plant-derived compounds have been linked to variations in testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), suggesting potential effects on the hypothalamic-pituitary-gonadal axis [9,10]. Changes in testicular antioxidant enzymes, lipid peroxidation and cellular morphology with large dosages of plant extracts have been documented in other investigations. Such findings emphasise the necessity of studying the positive and potentially hazardous impacts of traditionally used plants and their products.

Kola nut is widely used in Nigeria and other countries in West Africa however evidence on the effects of high dosage kola nut ethanolic extract on biochemical indices of testicular function is still relatively scarce. In particular, it is important to investigate whether long term exposure to high concentration of kola nut extract causes significant changes in testicular ACP and LDH activity. Such information may improve our knowledge of the possible reproductive effects of excessive kola nut consumption. This study was therefore conducted to determine the effect of high dosage kola nut ethanolic extract on testicular enzymes (acid phosphatase (ACP) and lactate dehydrogenase (LDH)) in male albino Wistar rats. The study aims to compare enzyme activities of control, low dose and high dose groups to find out the extent of significant biochemical alterations in testicular function with increasing dosage of kola nut ethanolic extract and to give experimental evidence of the potential reproductive risk of excessive kola nut intake.

MATERIALS AND METHODS

Study Area

The study was carried out at Madonna University Teaching Hospital, Elele, Rivers State, Nigeria. Elele is a town in Ikwerre Local Government Area of Rivers State, located in the South-South geopolitical zone of Nigeria. The area lies within the tropical rainforest ecological zone and experiences a tropical climate characterized by relatively high temperature and humidity throughout most of the year. The mean daily temperature is approximately 29°C, while annual rainfall is generally high, ranging from approximately 217 to 240 cm. Elele is surrounded by several towns and communities, including Isiokpo, Omagwa, Ahoada, Omoku and other settlements within Rivers State, with Owerri in neighbouring Imo State. The major occupations of the inhabitants include farming, trading and other commercial activities. The study area was considered suitable for the research because of the availability of the teaching hospital and laboratory facilities required for animal experimentation and biochemical analysis.

Research Design

The study employed a randomized controlled experimental research design to evaluate the effects of kola nut ethanolic extract on selected testicular enzymes in adult male albino Wistar rats. The animals were randomly allocated into three groups: a control group, a

low-dose treatment group and a high-dose treatment group. The treatment groups received kola nut ethanolic extract orally at doses of 100 mg/kg and 400 mg/kg body weight, respectively, for four weeks. At the end of the experimental period, the animals were sacrificed, the testes were collected and processed, and the activities of testicular acid phosphatase (ACP) and lactate dehydrogenase (LDH) were determined using spectrophotometric methods.

Preparation of Kola Nut Ethanolic Extract

Fresh kola nuts were purchased from a local market and transported to the Department of Botany for botanical identification and authentication. The nuts were thoroughly washed with clean water to remove adhering dirt and contaminants. They were subsequently peeled, sliced and the seed coats removed. The prepared kola nut samples were oven-dried at 40–50°C until a constant weight was obtained. The dried samples were then allowed to cool and were ground into a fine powder using a laboratory grinder.

A measured quantity of the powdered kola nut was extracted using 70% ethanol at a ratio of 1:10 (weight/volume). The powdered sample was soaked in the ethanol solvent at room temperature for 24 hours with intermittent stirring to facilitate extraction of the bioactive constituents. Following extraction, the mixture was first filtered through clean muslin cloth and subsequently through Whatman No. 1 filter paper to remove particulate materials.

The resulting filtrate was concentrated under reduced pressure using a rotary evaporator to remove the ethanol and obtain the crude kola nut ethanolic extract. The concentrated extract was further dried at a low temperature using a water bath or desiccator until a suitable consistency was obtained. The dried extract was weighed, transferred into a clean airtight container and stored at 4°C in a refrigerator until required for administration to the experimental animals.

Experimental Animals and Acclimatization

Twelve (12) adult male albino Wistar rats were obtained from a certified laboratory animal breeding facility. The animals were transported to the animal house and allowed to acclimatize for 14 days before commencement of the experiment. During the acclimatization and experimental periods, the animals were maintained under standard laboratory conditions at a temperature of approximately 25 ± 2°C, with a 12-hour light/12-hour dark cycle.

The animals had free access to standard laboratory rat feed and clean drinking water ad libitum. The cages were adequately ventilated and maintained under hygienic conditions. The animals were observed daily for general appearance, feeding behaviour, activity and any signs of illness or adverse reaction throughout the experimental period.

Experimental Grouping and Administration

Following the acclimatization period, the 12 male albino Wistar rats were randomly assigned into three groups comprising four (4) animals per group (n = 4).

- Group I (Control): Four rats received standard rat feed and distilled water only throughout the experimental period.
- Group II (Low-dose group): Four rats received standard rat feed and kola nut ethanolic extract at a dose of 100 mg/kg body weight orally.
- Group III (High-dose group): Four rats received standard rat feed and kola nut ethanolic extract at a dose of 400 mg/kg body weight orally.

The kola nut ethanolic extract was appropriately reconstituted in distilled water and administered orally using an orogastric tube once daily for four weeks. The administration volume was adjusted according to the body weight of each animal and the concentration of the prepared extract. Based on the experimental administration protocol, the control animals received distilled water, while the low-dose and high-dose groups received approximately 1.3 mL and 3.0 mL of the prepared extract formulation per rat, respectively.

The animals were monitored throughout the treatment period for changes in body weight, behaviour, food and water consumption, physical activity and any apparent signs of toxicity.

Sample Collection and Preparation

At the end of the four-week experimental period, the animals were fasted where appropriate and subsequently humanely sacrificed in accordance with approved laboratory animal procedures. The abdominal region was opened, and the testes were carefully dissected out. The tissues were freed from adhering connective tissue and blood, gently rinsed with appropriate physiological solution and blotted dry.

The testes were weighed and immediately processed for biochemical analysis. Each tissue sample was homogenized in an appropriate cold buffer solution to obtain a uniform tissue homogenate. The homogenates were centrifuged at an appropriate speed and duration, and the resulting supernatants were carefully collected and stored under suitable conditions until analysis of testicular enzyme activities.

Ethical Approval

Ethical approval for the study was obtained from the Madonna University Teaching Hospital Animal Ethics Committee with reference number MUN/VC/REC/MUN/MLS/21/2539. All experimental procedures involving the animals were conducted in accordance with the approved institutional protocol and internationally accepted guidelines for the care and use of laboratory animals. Efforts were made throughout the

study to minimize animal distress, discomfort and unnecessary suffering.

Determination of Testicular Enzymes

Lactate dehydrogenase activity was determined using an optimized ultraviolet (UV) spectrophotometric method

Principle

Lactate dehydrogenase (LDH) catalyses the reversible conversion of lactate to pyruvate, with nicotinamide adenine dinucleotide (NAD^+) serving as a coenzyme. In the reverse reaction, pyruvate is converted to lactate while NADH is oxidized to NAD^+. The change in absorbance resulting from the oxidation of NADH is measured spectrophotometrically at 340 nm. The rate of change in absorbance is proportional to the activity of LDH present in the test sample.Testicular tissue homogenate or its supernatant was used for the assay, and the enzyme activity was calculated according to the manufacturers or validated laboratory protocol for the analytical reagent used.

Acid phosphatase activity was determined using a colorimetric spectrophotometric method based on the hydrolysis of p-nitrophenyl phosphate (pNPP).

Principle

Acid phosphatase catalyses the hydrolysis of p-nitrophenyl phosphate (pNPP) in an acidic medium to produce p-nitrophenol and inorganic phosphate. Following incubation, sodium hydroxide is added to terminate the enzymatic reaction and convert p-nitrophenol into its yellow ionized form. The intensity of the yellow colour produced is measured spectrophotometrically at 405 nm. The absorbance obtained is directly related to the amount of acid phosphatase activity present in the test sample. The ACP activity in the testicular tissue homogenate was determined according to the manufacturer's instructions or validated laboratory protocol for the reagent kit employed.

Statistical Analysis

Data generated from the study were entered, organized and analysed using IBM SPSS Statistics version 27.0. Results were expressed as mean ± standard deviation (SD). Differences in mean testicular ACP and LDH activities among the three experimental groups were evaluated using one-way analysis of variance (ANOVA). Where the ANOVA indicated a statistically significant difference, Tukey's post hoc multiple-comparison test was used to determine the specific groups responsible for the observed differences. A P-value < 0.05 was considered statistically significant.

RESULTS

Table 1: Analysis of Variation (ANOVA) showing the mean Acid phosphatase and Lactate dehydrogenase activities among different groups fed with kola nut extract

Groups	ACP Activity (U/L)	LDH Activity (U/L)
1	227.00 ± 3.37	13.25 ± 1.71
2	230.50 ± 2.65	17.75 ± 1.26
3	233.33 ± 3.11	25.5 ± 2.89
F - value	4.536	35.903
P -value	0.043	0.000

Significance at p< 0.05; No- significance at p>0.05

Table 1 shows the Acid phosphatase (ACP) and Lactate dehydrogenase activities among different groups fed with kola nut extract. The control group exhibit mean ACP activity of 227.00 ± 3.37 U/L and LDH activity 13.25 ± 1.71s U/L. The low dose groups had ACP activity of 230.50 ± 2.65U/L and LDH activity of 17.75 ± 1.26 U/L. The high dose groups had ACP activity of 233.33 ± 3.11U/L and LDH activity of 25.5 ± 2.89U/L. The F-value of ACP was 4.536 with a P- value of 0.043 indicating significant difference. The F-value of LDH was 35.903 with a P- value of 0.000 indicating significant difference.

Post HOC Comparison

Table 2: Showing the multiple comparison of Acid phosphatase activity (ACP) and Lactate dehydrogenase activity (LDH) among different groups fed with kola nut extract.

Groups	ACP	LDH
Group 1 vs Group 2	0.287	0.032*
Group 1 vs Group 3	0.036*	0.000*
Group 2 vs Group 3	0.386	0.001*

Table 2 shows the multiple comparison of Acid phosphatase (ACP) and Lactate dehydrogenase (LDH) activity among different groups feed with kola nut extract. The asterisk (*) indicates statistically significant at 5% level for the groups are less than 0.05 (p<0.05) while groups without asterisk (*) are not significant.

DISCUSSION

This study was conducted to examine the effect of kola nut ethanolic extract on several testicular enzymes in male albino Wistar rats with special reference to acid phosphatase (ACP) and lactate dehydrogenase (LDH) as biochemical indicators of testicular cellular and metabolic activity. Results showed a progressive rise in the activity of both enzymes following administration of kola nut ethanolic extract with the most significant changes found in the high dose group. The control group had an activity of 227.00 ± 3.37 U/L for ACP and 13.25 ± 1.71 U/L for LDH. The low dosage group had an activity of 230.50 ± 2.65 U/L and 17.75 ± 1.26 U/L correspondingly. The highest activity was seen in the high dose group with ACP and LDH values of 233.33 ± 3.11 U/L and 25.50 ± 2.89 U/L respectively. These results indicate that exposure to kola nut ethanolic extract, especially at the higher dose, was related to variations in testicular enzyme activity. A statistically significant difference was seen in ACP activity across the experimental groups ($F = 4.536$, $P = 0.043$) indicating the effect of kola nut ethanolic extract on ACP activity. Acid phosphatase is involved with lysosomal activity and intracellular hydrolysis and changes in its activity may be indicative of changes in cellular metabolism or membrane integrity. The increased ACP activity in the treated groups, especially at the higher dose, may therefore imply increased cellular turnover, lysosomal activity or cellular stress in the testicular tissue [11]. However, enzyme increase alone is not sufficient to prove unequivocal structural tissue damage and should best be evaluated together with histological and other biochemical data. Likewise, in experimental groups, a significantly significant increase in activity of LDH was observed ($F = 35.903$, $P < 0.001$). LDH is a key enzyme in glycolytic energy metabolism and is found in several organs including the testes, in which energy production is critical for germ cell development and maturation. The significant elevation of LDH activity in the high dose group may be a sign of a change in energy metabolism in the testes or greater cellular stress. Increased levels could potentially be compatible with impaired cellular integrity, as LDH is released or changed following cellular damage [12]. The level of change in LDH activity in relation to control group indicates that LDH might be more sensitive to the effects of high dose of kola nut ethanolic extract. The higher alterations seen in the high dose group compared with the low dose group indicates a probable dose related response to kola nut ethanolic extract. ACP increased gradually from 227.00 U/L in control group to 230.50 U/L in low dosage group and 233.33 U/L in high dose group. LDH showed a more substantial rise with the values increasing from 13.25 U/L in the control group to 17.75 U/L and 25.50 U/L in the low and high dose groups respectively. This trend shows that with the increase of exposure to the extract, more modifications in testicle biochemical activity may be produced [13]. The observation is especially noteworthy because kola nut contains biologically active substances such as caffeine, theobromine, tannins and other phytochemicals, which could have more noticeable physiological effects at higher concentrations. One of the mechanisms of the observed changes may be oxidative stress. Overexposure to bioactive substances may disrupt the balance of reactive oxygen species (ROS) production and antioxidant defense mechanisms. The testes are particularly sensitive to oxidative damage due to the high metabolic activity and the high content of polyunsaturated fatty acids in the membranes of testicular and sperm cells. Elevated oxidative stress can lead to lipid peroxidation, modification of cellular membranes, and interruption of normal cellular activities. Such disruptions may therefore affect lysosomal activity and glycolytic metabolism, which could account for the alterations in ACP and LDH activities [14]. The results of the present investigation are in agreement with earlier publications that connect oxidative stress and exposure to high quantities of biologically active chemicals to abnormalities in testicular function [15]. Oxidative stress has been associated with changes in spermatogenesis, cellular membrane integrity, and reproductive function. Furthermore, it has been noted that consumption of excessive amounts of caffeine-containing products would affect metabolic and physiological pathways which could serve as a plausible explanation for the biochemical alterations that occur after high dose kola nut administration [16]. Caffeine is one of the key elements of kola nut and the observed effects cannot be ruled out due to caffeine. The increase in ACP activity could be associated with modifications in lysosomal activity and cellular turnover, whereas the increase in LDH might represent changes in energy metabolism and cellular integrity. Such changes may have ramifications for testicular function, if exposure is intense or long enough. Normal spermatogenesis requires a well-regulated cellular metabolism and an undamaged testicular microenvironment. Thus, prolonged biochemical abnormalities can interfere with the physiological processes essential for normal germ-cell growth. However, the present investigation was focused on the measurement of enzyme activity and did not directly examine sperm characteristics, reproductive hormones, markers of oxidative stress or testicular histology [17]. Thus, these data should be regarded as evidence of biochemical modification rather than conclusive confirmation of infertility or irreparable testicular injury. The data found may also have consequences on the safety of excessive use of kola nut. Kola nut is of great cultural and traditional significance and may have stimulating effects in modest quantities. The present results show that high dose exposure can change the testicular biochemical markers in experimental animals. This finding highlights the need for further investigations on the consequences of repeated or chronic exposure to large doses of kola nut components. However, it is important to acknowledge the differences between experimental animals and humans and not to equate the doses employed in animal

trials directly with similar human consumption without sufficient pharmacological and toxicological assessment [18].

CONCLUSION

The present study results demonstrated that treatment of kola nut ethanolic extract induced significant changes in testicular ACP and LDH activities in male albino Wistar rats. The activities of both enzymes were higher in the high-dose group. The activities of ACP and LDH increased progressively compared with the control and low-dose groups. The large increase in ACP and LDH suggests that high dose of kola nut ethanolic extract can influence cellular activity, lysosomal function and energy metabolism in the testis and may be linked to cellular stress or tissue integrity compromise. The more dramatic effects reported at 400 mg/kg body weight further indicate the possibility of a dosage related impact. However, given this study was confined to biochemical measures of the enzymes, the observed increases in enzymes should not be taken as proof of irreversible testicular injury or infertility. Further studies incorporating testicular histopathology, sperm quality, reproductive hormone profiles and oxidative stress measures are suggested to establish the underlying mechanisms and to determine the wider reproductive consequences of high dosage kola nut ethanolic extract exposure.

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