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Reproductive Toxicity of *Quassia amara* Extract: Action on Sperm Capacitation and Acrosome Reaction

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Abstract: *Quassia amara* is a medicinal plant with several pharmacological properties. There are preliminary reports implicating this plant in male reproductive toxicology. Single daily oral administration of methanol extract of *Quassia amara* stem bark for 6 weeks resulted in a significant reduction in the weight of the testes, caudal epididymis and seminal vescicle. Caudal epididymal sperm parameters showed evidence of toxicity. The sperm motility, viability, count and volume were significantly reduced ($P<0.05$). Less than 50% of the caudal epididymal sperm were motile. Total tissue protein of the epididymis of the treated rats was significantly reduced ($P<0.05$), but no significant difference was recorded in the testicular total tissue protein when compared with the control. Luteinizing hormone and testosterone levels were significantly lower ($P<0.01$), but no effect was observed on follicle stimulating hormone. Histological section showed that the epididymis is made up of fewer ducts, which are loosely packed together when compared with the control and the ducts contained less amounts of the ductular eosinophilic material. The ductular epithelium however remained normal. No visible lesion was observed in testicular epithelium. *In vitro* study to verify these results on caudal epididymal sperm of white Fulani cow in an extender solution showed that the reproductive toxic effect of *Q. amara* is consistent. Sperm parameters declined in the treated groups. Ninety seven percent of the caudal epididymal sperm of the control rats were capacitated and their acrosome reacted after *in vitro* induction of sperm capacitation by pre-incubation in a modified capacitation medium. However, thirty nine percent of sperm cells from rats treated with *Q. amara* failed to be capacitated and their acrosome remained intact while thirty three percent failed to undergo the acrosome reaction, though they were capacitated. Only 27% successfully underwent the capacitation and acrosome reactions. *Q. amara* may have a reproductive specific toxic effect as our results show lack of effect on weight and histology of visceral organs, fasting blood glucose level, lipid profile and liver function.

Key words: *Quassia amara* · Sperm · Caudal epididymis · Capacitation · Acrosome · Hormone

INTRODUCTION

The role of epididymis in sperm functions such as sperm survival, maturation, motility, capacitation and fertilizing ability cannot be overemphasized. The molecular basis of these functions is beginning to be elucidated in recent time with the discovery of some epididymis-specific proteins and their functions. Some of the newly discovered proteins include Bin1b (found in different regions of the epididymis) which is important for the acquisition of sperm motility and the initiation of sperm maturation [1], Rnase9 (localized mainly in caput), which is important for sperm maturation [2], Hongr ES1 (found in cauda), which is important in regulation of sperm capacitation and male fertility [3] and Glb1l4, which is an essential protein in the caput epididymis and is important in epididymal development and sperm maturation in rats [4]. This major discovery has opened an important window of opportunity for research into the development of an effective non-steroidal male contraceptive agent using epididymis as a target organ.

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Quassia amara (bitter wood) is a potential candidate for male contraceptive development. Extract of *Q. amara*, a plant with strong antimalarial and antimicrobial activities was reported to have strong male antifertility actions in rats and mice [5-8]. Quassin, a bioactive compound isolated from *Q. amara* was implicated as the male antifertility principle.

The major site of action of quassin appears to be the reduction in sperm motility, count and morphology. These activities may indicate its probable action at the epididymal level for which the molecular basis has not been explored. To date there is no information on the action of *Q. amara* on sperm capacitation. This study was therefore designed to investigate the action of *Q. amara* on sperm capacitation as a prelude to a detailed investigation of its molecular mechanism of action on protein expressions in the epididymis.

MARETIALS AND METHOD

Experimental Animals: Adult male Wistar strain albino rats (180-200g) were housed in well-ventilated animal cages in the central animal house, college of medicine, University of Ibadan with constant 12-h light 12-h dark cycle. They were fed standard rat feed and clean water *ad libitum* and were allowed two weeks of acclimatization.

All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and Use of animals [9].

Preparation of Extract: The stem bark of *Q. amara* was obtained from the Botanical Gardens, University of Ibadan and was authenticated at herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher number 109103 was assigned to the specimen of *Q. amara*. The air-dried stem bark (2.51 kg) was soaked in aqueous methanol at room temperature for 5 days with daily shaking and thereafter was decanted. The extract obtained was concentrated to a dark-green residue (24.4 g) on a rotary evaporator at 40°C and weighed. Percentage yield was 0.97%.

Experimental Design: *Quassia amara* stem bark extract (100mg kg^{-1} b.w.) was administered daily (p.o.) for 6 weeks to an experimental group while the control group received vehicle (distilled water) for same duration. Each group was made up of 5 male rats. At the end of 6 weeks of treatment, all male rats were sacrificed by decapitation. Reproductive and visceral organs were excised, cleared of fat and connective tissue and weighed to the nearest milligram. Tissues and visceral organs for histological studies were preserved in Bouin's solutions while reproductive organs were stored in phosphate buffered saline (PBS, pH 7.4) at -20°C for estimation of total protein.

Sperm Analysis: Sperm characteristics analysis was performed on spermatozoa samples collected from the caudal epididymis using Olympus research microscope (Olympus, Japan) under x40 microscope objectives. Progressive motility was assessed immediately. Five-microlitre drop of diluted sperm suspension was placed on a pre-warmed slide and two drops of warm 2.9% sodium citrate was added and covered with cover slip. Progressive forward motility was examined and scored to the nearest 10 [10]. Viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and unstained sperm cells were counted and an average value for each was recorded from which percentage viability was calculated. Sperm count was done under the microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers [11]. The epididymis was immersed in 5 ml normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis [12].

Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewa stain after they were air-dried. The slides were examined under the microscope under oil immersion with x 100 objectives. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce [13].

Estimation of Total Tissue Protein: Total protein was estimated spectrophotometrically according to the method described by Lowry *et al*. [14] using PBS as standard. Briefly, epididymis and testes were washed in ice cold 1.15% KCl solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in four volumes of the homogenizing buffer (PBS, pH 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 10,000rpm for 10mins in a cold centrifuge (4°C) to obtain post mitochondrion fraction. The supernatant was collected and total protein estimated using Randox total protein kit (Randox Laboratories Ltd, United Kingdom).

Determination of Fasting Glycemia (G0): Fasting glycemia was estimated after 6 weeks of extract treatment using glucose oxidase method as described by Barham and Trinder [15].

Hormone Assay: An enzyme–based immunoassay (EIA) system was used to measure FSH, LH and testosterone levels in serum samples collected. The EIA kit was obtained from immunometrics (London, UK) and contained the respective EIA enzyme label, EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity of approximately 0.022ng/mL of testosterone. The intra and inter assay variations for testosterone were 10.02 and 10.12% respectively.

Biochemical Assays: The serum levels of alkaline phosphatase (ALP), alanine aminotrasferases (ALT) and aspartate aminotransferases (AST) were assayed by the method of Moss and Henderson [16]. Total cholesterol, triglyceride, high-density lipoproteins (HDL) and low-density lipoprotein (LDL) were measured as described by Rifai *et al*. [17]. All the biochemical parameters were assayed using the respective commercial diagnostic kits obtained from Diasys Diagnostic systems (Istanbul, Turkey) on a Statfax Diasys 1904 plus Biochemical Analyzer.

Sperm Preparation and Capacitation: Sperm specimens were collected by (teasing) perfusing the caudal epididymis of rats through the distal end of the vas deferens into a pre-warmed modified sperm capacitation medium (SCM). The SCM was modified from that of Toyoda and Chang [18] and contained 110 mM NaCl, 2.7 mM KCl, 2.4 mM $CaCl_2$, 0.49mM $MgCl_2$, 0.32 mM NaH_2PO_4, 24.9 mM $NaHCO_3$, 5 mM Glucose, 6.26 mM Lactate, 0.125 mM pyruvate and 4 mg/ml Bovine Serum Albumin (BSA; pH 7.5). Capacitation of rat sperm was induced by the modified method of Feng *et al*. [19]. Sperm concentration was determined using an improved Neubauer haemocytometer to adjust to 2×10^6 sperm/ml. One hundred microlitre (100µl) of sperm sample was transferred to an eppendorf tube containing 1ml of SCM. This was done in triplicate. Suspension specimen was then incubated for 3 hours at 37°C in a moist atmosphere of 5% CO_2. These conditions are known to induce sperm capacitation and acrosome reaction [20-22]. Aliquots of sperm were removed from each group for assessment. Sperm motility and movement characteristics were assessed at the end of the incubation with the aid of microscope at room temperature. Sperm acrosomal status was then assessed using coomassie brilliant blue staining technique [19].

Acrosome Staining Using Coomasie Brilliant Blue: Sperm samples were air-dried on glass slides and fixed with ethanol. After drying, slides were then immersed in a solution of 5% paraformaldehyde in PBS for 15minutes and washed once with PBS. Slides were then stained with aqueous 0.25% CBB R-250 (Sigma chemical company, USA) in 10% glacial acetic acid and 25% methanol. They were then rinsed with clean water, air dried and covered with cover slips under mounting media (Olympus, Japan). Acrosome region is stained blue in the acrosome-intact sperm but unstained in the acrosome-reacted sperm [19]. A minimum of 100 sperm cells in each sample were classified as expressing one of three staining patterns: 'A', with uniform (head and acrosome) staining which is characteristic of acrosome intact uncapacitated sperm; 'B', with staining in the post acrosomal region which is characteristic of acrosome intact capacitated sperm and 'C', with absent head staining which is characteristic of acrosome-reacted capacitated sperm cells [23].

***In vitro* Extender Study:** The caudal epididymis of a freshly sacrificed white Fulani cow was excised. Blood clot and other tissues were rinsed away using sodium citrate solution. Sperm cells were collected from the caudal epididymis by teasing. Pre-extension motility was determined by mixing one drop of the sperm cells with one drop of 2.9% sodium citrate and observed under the light microscope with X40 objective.

The extender solution was composed of 80% buffer (trisodium citrate) and 20% egg yolk. The freshly collected sperm cells was carefully aspirated to the 1ml of a syringe and then dispersed into a 50ml conical flask containing the extender solution. The mixture gives an extension rate of 1:50. The mixture was then gently shaken and 5ml was dispersed into series of test tubes each.

Graded doses (50-500 µg/ml) of crude methanol extract of *Q. amara* were then added to the series of extender solutions. Sperm motility, viability and morphology were observed and recorded immediately and thereafter hourly over a period of four hours.

Statistical Analyses: Data were expressed as mean±standard error of mean (SEM). The test of significance between two groups was estimated by student's t-test. P<0.05 was considered significant.

RESULTS

There were no changes in animal behavior or body weight during treatment with *Q. amara* stem bark (Table 1). Also, treatment had non-significant effect on the visceral organ *viz.,* lungs, liver, heart and kidney weights (Table 2). Most of the sperm of the control rats had normal counts, motility and morphology. However, the weight of the testes, caudal epididymis and seminal vescicle was significantly lower (P<0.05) in the treated group, when compared with the control (Table 3). Caudal epididymal sperm parameters showed evidence of toxicity when compared with the control (Table 4). The sperm motility, viability and count were significantly reduced (P<0.05). Less than 50% of the caudal epididymal sperm were motile. Also, the semen volume was significantly reduced (P<0.05) and percentage of sperm cells with abnormal morphology was higher (P<0.05).

In-vitro studies to verify these results showed that graded doses (50-500 µg/ml) of methanol extract of *Q. amara* stem bark decreased sperm motility (Table 5) and viability (Table 6) in an extender solution of sperm cells collected from the caudal epididymis of a white Fulani cow in a dose dependent manner. Also, the percentage of sperm cells with abnormal morphology increased with treatment dose (Tables 7).

Histopathological examination of the treated rats revealed that the epididymis was made up of fewer ducts, which were loosely packed together when compared with the control and the ducts contained less amounts of the ductular eosinophilic material.

Table 1: Total body weights (g) of experimental rats treated with *Q. amara* extract and corresponding control group. No significant changes were observed

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	SACRIFICE
Control	190±4.5	200±5.5	220±8.9	224±7.5	244±6.8	246±4	260±7.1
Treated	200±0	224±4	248±4.9	244±8.1	256±11.7	248±12.4	258±16.9

Values are mean±SEM, n = 5, g = gram

Table 2: Visceral organ weights (g) of experimental rats treated with *Q. amara* extract and corresponding control group

Organ	Lungs (g)	Liver (g)	Heart (g)	Kidney (g)
Control	1.48±0.04	6.54±1.31	0.6±0.09	0.68±0.05
Treated	1.48±0.1	8.1±0.3	0.68±0.05	0.72±0.02

Values are Mean±SEM, n = 5, g = gram. Comparisons made with the values of the corresponding control group (student's t-test) showed no significant changes.

Table 3: Effects of *Q. amara* extract on weight (g) of sex and accessory sex organs

Group	Testes (g)	Caudal Epididymis (g)	Seminal Vescicle (g)
Control	1.18± 0.02	0.30±0	1.6±0
Treated	0.78±0.19 *	0.15±0.02 *	0.9±0.21 *

Mean ±SEM, n = 5, g = gram, * P<0.05 indicates significant difference from control

Table 4: Effects of *Q. amara* extract on Sperm parameters

Group	Motility (%)	Viability (%)	Count (million/ml)	Volume(ml)	Abnormal Morphology (%)
Control	93.75±1.25	97.25±0.75	120.49±5.40	5.20±0	10.49±0.34
Treated	42.5±13.15 *	87.5±3.23 *	58±5.48 *	5.15±0.004*	15.61±1.13 *

Mean±SEM, n = 5, * P<0.05 indicates significant difference from control

Table 5: Effect of *Q. amara* extract on sperm motility *in vitro*

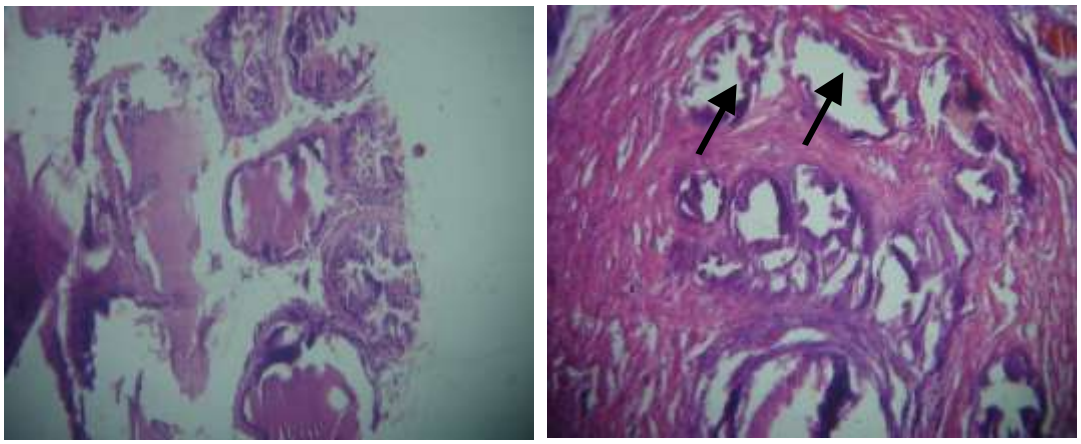
Group	0 Hrs	1Hr	2Hrs	3Hrs	4Hrs
Control	90	90	90	80	80
50 µg	90	80	80	70	70
100 µg	80	80	80	80	70
150 µg	80	70	80	70	60
200 µg	80	80	70	70	70
250 µg	70	60	50	40	40
500 µg	60	40	20	10	10

Table 6: Effect *Q. amara* extract on sperm viability (live/dead ratio) *in vitro*

Group	0 Hrs	1Hr	2Hrs	3Hrs	4Hrs
Control	98	98	98	95	95
50 µg	98	90	85	80	80
100 µg	95	95	90	85	80
150 µg	95	85	85	80	80
200 µg	95	90	85	80	80
250 µg	95	95	90	85	80
500 µg	80	80	70	70	70

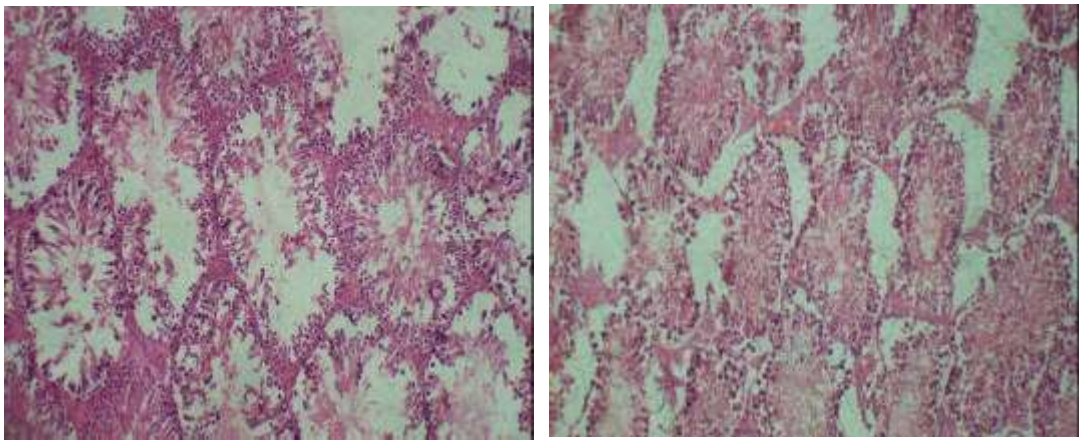
Table 7: Effect *Q. amara* extract on sperm morphology *in vitro*

Group	Control	50 µg	100 µg	150 µg	200 µg	250 µg	500 µg
Abnormal Morphology (%)	8.67	9.51	9.51	11.11	11.08	11.95	16.86



Control Extract Treated

Fig. 1: Transverse sections through the Epididymis of normal control rats and rats treated with 100mg/kg *Q. amara* stem bark extract. Arrow indicates loosely packed epididymal ducts in the treated group when compared with control.



Control Extract Treated

Fig. 2: Transverse sections through the Testes of normal control rats and rats treated with 100mg/kg *Q. amara* stem bark extract. No visible lesion is observed when compared with the control

The ductular epithelium however remained normal (Figure 1). However, no visible lesion was observed in the testes (Figure 2). Transverse section through the seminal vesicles of the treated rats shows erosion of the epithelial membrane (Figure 3). Examination of the visceral organs (lungs, liver, heart and kidney) showed that treatment had no effect on the histology of these organs (photomicrograph not shown).

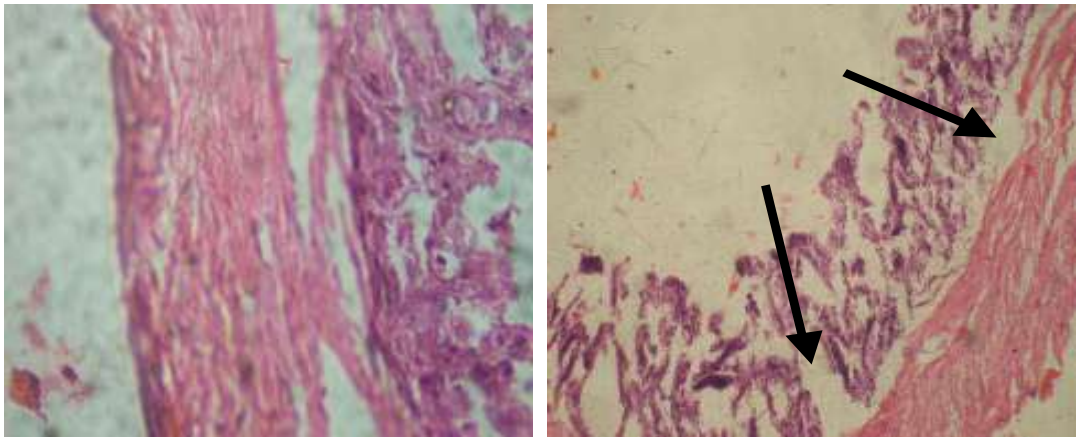


Fig. 3: Transverse sections through the Seminal vescicle of normal control rats and rats treated with 100mg/kg *Q. amara* stem bark extract. Arrow indicates epithelial erosion of the treated group when compared with control.

Table 8: Effect of Q. *amara* extract on Epididymal and Testicular total protein

Group	Epididymal Total Protein (g/dl)	Testicular Total Protein (g/dl)
Control	1.65±0.32	0.88±0.14
Treated	0.67±0.11*	1.37±0.17

Values are Mean±SEM, n = 5, * P<0.05, indicates significant difference from control

Table 9: Effect of *Q. Amara* on Serum Gonadotropin and Testosterone Levels

Group	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
Control	4.42±0.47	1.5±0.18	0.32±0.09
Treated	5.36±0.35	0.6±0.14**	0.03±0.01**

Values are Mean±SEM, n = 5, ** P<0.01, indicates significant difference from control

Table 10: Effect of *Q. Amara* on Serum Lipid Profile

Group	T Chol (mmol/l)	Tg (mmol/l)	LDL (mmol/l)	HDL (mmol/l)
Control	1.38±0.14	0.46±0.13	0.7±0.07	0.58±0.08
Treated	1.34±0.12	0.42±0.08	0.7±0.04	0.32±0.08

Values are Mean±SEM, n = 5. Comparisons made with the values of the corresponding control group (student's t-test) showed no significant changes.

Table 11: Effect of *Q. Amara* on Liver Function

Group	ALP (U/L)	ALT (U/L)	AST (U/L)
Control	144.6±19.9	11.8±3.77	14.0±1.76
Treated	92.4±15.0	18.8±2.69	12.2±1.53

Values are Mean±SEM, n = 5. Comparisons made with the values of the corresponding control group (student's t-test) showed no significant changes.

Table 12: Fasting glycemia (G0) of experimental rats treated with *Q. amara* extract and corresponding control group.

Group	G0 (mg/dl)
Control	80±9.3
Treated	101.4±4.5

Values are Mean ±SEM, n = 5. Comparisons made with the values of the corresponding control group (student's t-test) showed no significant changes.

Table 13: Patterns of sperm expression after incubation in a sperm capacitation medium

Group	Acrosome-intact uncapacitated sperm	Acrosome-intact capacitated sperm	Acrosome reacted capacitated sperm
Control	2.6±0.5	2.2±0.58	95.2±0.86
Treated	39.4±3.97**	33.4±3.12**	27.2±1.98**

Values are Mean±SEM. ** P<0.01, indicates significant difference from control

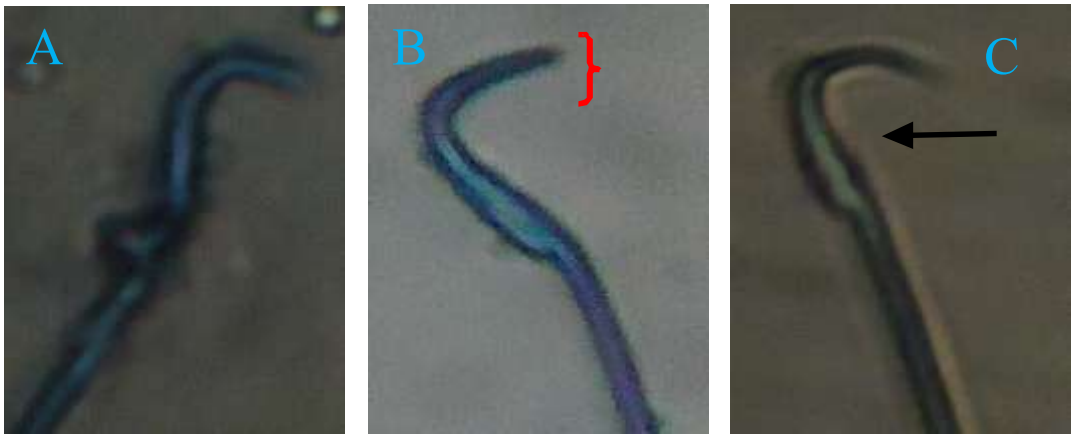


Fig. 4: Coomassie brilliant blue (CBB) staining patterns of rat spermatozoa during capacitation. A. Uncapacitated cell with uniform head staining. B. Acrosome intact capacitated sperm with staining in the acrosomal region and C. Acrosome-reacted capacitated sperm cells with no head staining (× 400 Magnification).

Table 8 showed that *Q. amara* stem bark extract significantly decreased the epididymal total tissue protein ($P<0.05$), but no significant difference was observed in the esticular total tissue protein when compared with the control.

Serum levels of LH and testosterone were significantly lower ($P<0.01$) in the treated group when compared with the control (Table 9). However, no significant difference was observed in the FSH level. Also, biochemical parameters to assess the serum lipid profile (Table 10) and liver toxicity (Table 11) were not affected by *Q. amara* treatment. There was no significant change in fasting blood glucose in *Q. amara* treated rats when compared with the control (Table 12).

Over 97% of the caudal epididymal sperm of the control rats were capacitated and their acrosome reacted after *in vitro* induction of sperm capacitation by pre-incubation in a modified capacitation medium. However, 39%±3.97% of sperm cells obtained from rats treated with *Q. amara* failed to be capacitated and their acrosome remained intact. About 33%±3.12% failed to undergo the acrosome reaction, (although they were capacitated) while only 27%±1.98% of the sperm collected from the caudal epididymis were capacitated and their acrosome reacted (Table 13). All the uncapacitated sperm had their acrosome intact. Figure 4 shows the different staining patterns of the rat spermatozoa during capacitation.

DISCUSSION AND CONCLUSION

In this study, we investigated the effects of *Q. amara* stem bark extract on male reproductive parameters and more closely, its impact on the physiology of sperm cell. *Q. amara* significantly decreased the weight of testes, caudal epididymis and seminal vescicle. There was no difference in the total body and visceral organ weights of the treated group when compared with the non-treated control. This is consistent with the finding of Parveen *et al*. [7], which reported that *Q. amara* extract had no effect on hematological and serological paramaters.

Sperm acquire the capability for motility during their epididymal transit and in the normal course, all caudal epididymal sperm are motile [3]. Toxicity effect of *Q. amara* stem bark extract on caudal epididymal sperm motility, viability, count, volume and morphology shown in our results demonstrate that the extract interferes with the structural integrity and functional competence of the sperm cells *in vivo*. This is consistent with earlier reports in literature [6-8].

In vitro studies to verify these results on caudal epididymal sperm of white Fulani cow in an extender solution showed that the reproductive toxic effect of *Q. amara* is consistent and increased in a dose dependent fashion. Comparatively, the progressive motility declined immediately from 90% in the control group to 60% in the group treated with 500 µg/ml crude *Q. amara* extract. Over a period of four hours in the extender solution, live/dead ratio of the epididymal sperm remained almost unchanged (Table 6) and only a 10% decline in motility (Table 5) was observed in the control group. This shows that the extender solution physiologically preserved sperm survival within the four-hour study duration post extension. Slight decline in sperm motility was observed at lower doses of treatment up to 200µg/ml, with only an average of 10% decline in motility over four-hour study duration. Motility declined from 70 to 40% when treated with 250 µg/ml and from 60 to 10% when treated with

500 µg/ml *Q. amara* extract. Also the sperm viability declined slightly with treatment *in vitro*. More than 95% of the control sperm cells were alive after four hours in an extender solution, while about 70% was alive in the group treated with 500 µg/ml *Q. amara* extract (Table 6). The percentage of sperm cells with abnormal morphology however increased in a dose dependent manner (Table 7).

The contributory factors to the initiation of spermatozoa motility, mainly in the form of proteins and small molecular weight glycoproteins emanate from the epididymal epithelia cells [3]. Derangement of the epididymal ducts and impairment of motility of caudal epididymal sperm of treated rats is a reflection of the effect of this extract on the physiologic anatomy of the epididymis [8]. The decrease in epididymal tissue total protein recorded in the treated group further emphasizes the toxicity effect of this extract on the epididymis.

Although no difference was recorded in the testicular tissue total protein, the decrease in sperm count (about 50%) and the increased number of morphologically abnormal sperm indicates interference with testicular spermatogenesis. The decline in testosterone and LH levels recorded after treatment further corroborates this assumption. Although no decline in FSH was observed in this study, the decline in LH, testosterone, sperm count and organ weight restricted to the sex organs suggest that *Q. amara* may act on the pituitary-gonadal axis. Raji and Bolarinwa [6] however reported a decrease in weight of the anterior pituitary and a decline in *in-vitro* testosterone production from isolated Leydig cells when intact rats were treated with the same extract and hypothesized that *Q. amara* site of action may include the pituitary gland and the Leydig cells.

The lack of alteration of fasting glycemia, serum lipid profile and hepatic intracellularly localized marker enzymes (such as ALP, ALT and AST) after *Q. amara* treatment further support the findings that this extract may have a reproductive specific toxic effect. This by extension may be inferred to mean lack of effect on pancreatic β-cells and insulin regulating mechanisms [24, 25] and lack of injury to liver hepatocytes [26].

To achieve successful fertilization under normal circumstances *in vivo*, mammalian spermatozoa must first undergo capacitation and then acrosome reaction [27, 28]. The extracellular environment play crucial role in achieving these complex events that permit spermatozoa to achieve fertilizing ability at the right time and in the right place [3]. Spermatozoa collected from the control rats showed more than 80% active progressive motility in every trial throughout the incubation period in the sperm capacitation medium, while about 40% of the spermatozoa of the treated rats were still motile at the end of incubation. Preincubation of spermatozoa of the control rats in the standard medium induced sperm capacitation in almost all the sperm cells with concurrent spontaneous acrosomal reaction.

Coomassie brilliant blue (CBB) staining is much more convenient and stable than other methods for evaluating acrosome reaction [29]. Using CCB staining technique, *Q. amara* treatment was shown to significantly decrease the number of capacitated sperm cells ($P<0.05$) and about 33% of the cells had their acrosome intact, despite being capacitated. Thus over 70% of the spermatozoa obtained from the caudal epididymis of the treated group were functionally impaired and may be incapable of fertilizing an ovum, as only 27% of these were capacitated and had their acrosome react.

Development of culture systems that support capacitation and fertilization *in vitro* has made it possible to determine ions required specifically for capacitation and acrosome reaction. Ca^{2+} has been proved to be the most important ion [30, 31]. Also Na^{+}, in low concentration has been proved to be a necessity for sperm capacitation [32, 33]. Capacitation is slow, involving internalization of Ca^{2+} over a period of hours [34, 35], while acrosome reaction involves rapid internalization of Ca^{2+} over few minutes [36]. Ca^{2+} channel blockers such as verapamil and nifedipine have been shown to block sperm capacitation and acrosome reaction [19]. *Q. amara* may mediate its antifertility action by acting on ion channels or on caudal epididymal sperm proteins critical for sperm capacitation. Further ongoing study in our Laboratory is expected elucidate the mechanism of action of *Q. amara*, showing its effect on ion channels and epididymis specific proteins such as HongrES1 and Bin1b which has been shown in literature to critically time sperm capacitation and the acrosome reaction [3].

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