MALE REPRODUCTIVE TOXIC EFFECT OF *QUASSIA AMARA*: OBSERVATIONS ON MOUSE SPERM

Faisal K[1], Parveen S[2], Rajendran R[3], Girija R[1], Periasamy VS[1], Kadalmani B[1], Puratchikody A[4], Ruckmani K[4], Pereira BMJ[2], Akbarsha MA[1]

[1] Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India.
[2] Department of Bioscience and Biotechnology, Reproductive Biology Laboratory, Indian Institute of Technology, Roorke 247 667, India.
[3] Green Chem, Attibele Industrial Area, Bangalore - 562 107, India.
[4] Department of Pharmaceutical Engineering & Technology, Bharathidasan Institute of Technology, Tiruchirappalli - 620 024, India.

SUMMARY

Quassia amara is a medicinal plant with several pharmacological properties. More recently, it has come to be known in the context of its potential biopesticidal application and the antimalarial property in two of its secondary metabolites, quassin and neo-quassin. There are also preliminary reports implicating this plant in male reproductive toxic effects, to the extent that the plant could as well be tested for male contraceptive efficacy. Therfore, we subjected the methanolic extract of the bark of this tree for male reproductive toxicological evaluation. This paper reports several derangements in the cauda epididymidal sperm including a hither - to unreported one.

Key words: Neo-quassin, *Quassia amara*, Quassin, sperm abnormalities.

INTRODUCTION

Quassia amara (Surinam Wood), belonging to the family Simaroubaceae, is a tree species naturally distributed in several tropical countries. Traditionally, the bark and leaves of this tree are used in herbal remedies, since they are rich in biologically active principles. Its leaves, bark and heartwood are attributed with pharmacological properties such as anti-malarial, anti-fungal, anti-ulcerative, anti-edimogenic and anti-cancer (1). Quassin and neo-quassin, the major secondary metabolites in this tree, are being extensively investigated for antimalarial property (2). Recently, anti-viral activity in the extract has also been proposed (3). However, the application of this plant in bio-control of insect pests, based on its insecticidal, larvicidal, pediculicidal and anti-feedant properties, has been recommended, and these effects should lie in some toxic property of this plant (4). The male reproductive system, particularly spermatogenesis, sperm maturation and androgen biosynthesis, are highly sensitive to toxic insults, and the properties in *Q. amara* which would be useful in insect pest control could affect the physiology of male reproduction in the non-target organisms. Such toxic effects could as well manifest in men who are treated with *Q. amara* or the secondary metabolites in it. Therefore, study of long-term toxic effects of the plant, when applied directly as a therapeutic or indirectly as a biopesticide, is pertinent. There are three preliminary reports implicating *Q. amara* in male reproductive toxic effects (5-7). In this background, an exhaustive study was initiated to find the male reproductive toxic effect of *Q. amara* in an experimental animal model, Swiss mouse. This note reports the sperm abnormalities in the treated mice.

MATERIALS AND METHODS

The bark of the tree was quantitatively extracted in methanol using a Soxlet apparatus and the solvent was evaporated in a rotary evaporator. The lyophilized methanol extract was quantitatively dissolved in a minimum quantity of ethanol and diluted with phosphate-buffered saline (PBS).

Twenty four 90 day old male mice (30-35 g body weight) were divided into 4 groups of 6 each. The common control group was administered with PBS, and the remaining groups were injected with the methanolic extract at a daily dose of 25, 50 and 100 mg/kg body weight through intraperitoneal route for 35 days (the duration of one spermatogenic cycle in mouse).

At the end of the experiment, the animals were dissected under sodium pentabarbital anaesthesia and the epididymes were removed. The cauda epididymidal spermatozoa were subjected to counts (using Neubauer counting chamber under appropriate dilution), motility (assessed using hanging drop preparations) and observation of morphology (using Giemsa's stained smears), in a Carl Zeiss Axio-Plus 2 research microscope (Carl Zeiss, Jena, Germany).

Tissue slices of the cauda epididymidis, fixed in 2.5% glutaraldehyde and post-fixed in 1% OsO_4 were

Correspondence to be addressed to: Prof. M. A. Akbarsha, Ph.D. Email: akbarbdu@yahoo.com

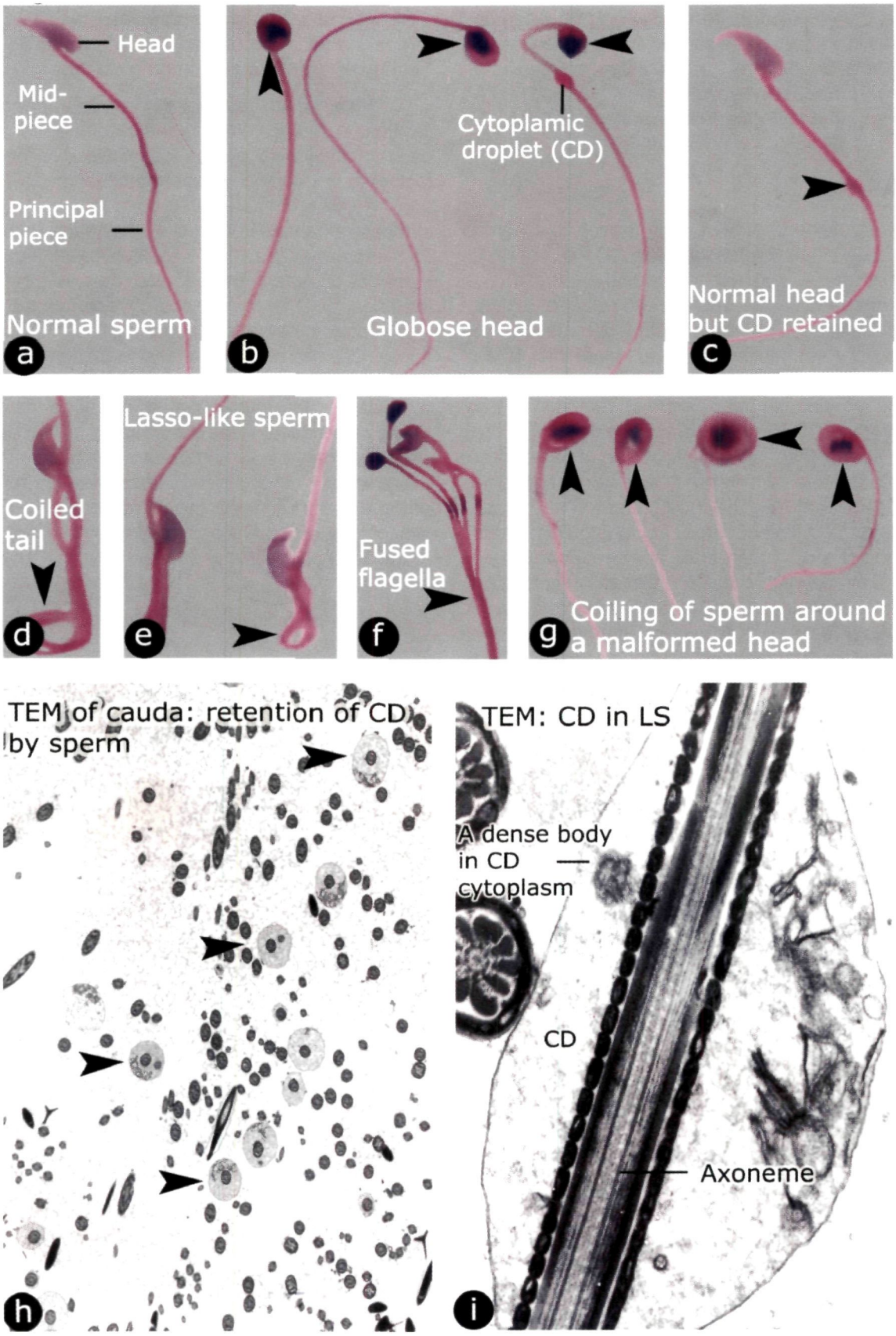

Fig.1. Sperm of control (a) and treated (b-i) mice. CD, Cytoplasmic droplet. The other details are indicated in the respective figures.

embedded in thin viscocity resin (Sigma Chemical Co., USA). Ultrathin sections were stained in uranyl acetate and lead citrate and observed in a Phillips transmission electron microscope (Amsterdam, Holland). The photomicrographs were image-processed using Adobe 7.0 software.

RESULTS

Most of the sperm of control mice had normal counts, motility and morphology (Table 1, Fig. 1a). In *Q. amara* methanolic extract-treated mice the cauda epididymidal sperm parameters showed evidence of dose-dependent toxicity. The sperm counts decreased. The sperm motility was inhibited (Table 1). More than 50% of the sperm had abnormal morphologies of various kinds, which included globose head (Fig. 1b), retention of cytoplasmic droplet (CD) (Fig. 1b, c), coiling of tail (Fig. 1d), lasso-like sperm (Fig. 1e), fusion of tails of two or more sperm (Fig. 1f), etc., which are abnormal morphologies known in the literature. The most common and unique abnormality was coiling of part or entire flagellum around a dense object which, apparently, is the malformed head (Fig. 1g). Low magnification TEM pictures revealed failure of cauda epididymidal sperm to shed the cytoplasmic droplet (CD) (Fig. 1h, i). A feature of interest in the cytoplasm of the CD, thus retained, was presence of a dense body in addition to the membrane elements characteristic of the CD.

Table 1. Sperm parametic analysis in *Q. amara* extract - treated mice (values, mean ± SD)

Treatment	Parameter		
	Counts (Million/ml)	Motility(%)	Abnormal sperm (%)
Control	6.8 ± 0.8	94.6 ± 4.8	04.16 ± 0.86
Q. amara 25 mg	4.2 ± 0.6*	68.4 ± 3.6*	48.42 ± 6.08*
Q. amara 50 mg	3.5 ± 0.5*	52.3 ± 2.8*	59.64 ± 6.42*
Q. amara 100 mg	1.8 ± 0.2*	34.6 ± 1.6*	66.36 ± 7.86*

* p=0.01 ; control vs treated

DISCUSSION

The data generated in this study, by and large, conform to those already reported (5 - 7). The decrease in the cauda epididymidal sperm counts and globose head are clear indications that *Q. amara* methanolic extract can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of *Q. amara* on the cellular mechanisms of spermatogenesis can not be exonerated, it is likely that the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis, already known in the literature (6, 7), may as well be the underlying cause.

The sperm acquire the capacity to motility during their epididymal transit, and in the normal course all cauda epididymidal sperm are motile. The contributory factors to the initiation of spermatozoal motility, mainly in the form of proteins and small molecular weight substances, emanate from the epididymal epithelial cells. The impairment of motility of the cauda epididymidal sperm of *Q. amara*-treated mice, thus, is a reflection of the effect of *Q. amara* on the physiology of epididymis. The various other sperm abnormalities, including retention of CD, are also indications to epididymis also as a target to *Q. amara* toxicity. Again, these effects may be attributed to the impairment of hormonal mechanisms already discussed, since the structure as well as function of the epididymis is dependent on hormones in the hypothalamo – hypophysial - testis axis. However, some of the abnormalities like coiled tail, lasso-like sperm, fusion of tails, etc., suggest a direct toxic effect of *Q. amara* at the level of epididymis (8, 9).

The hitherto un - reported abnormal sperm morphology, in the coiling of tail around an apparently malformed head, could be attributed to both testicular and epididymal effects of *Q. amara*. Coiling of the sperm tail is usually the product of abnormal axoneme and / or the outer dense fibrils, but may also suggest alterations in the sperm surface proteins .

The outcome of the study affirms the male reproductive toxic effects of *Q. amara* when applied as a therapeutic or as a bio-pesticide. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequences of *Q. amara* on the sperm may be taken to advantage for further study on this tree and its secondary metabolites for efficacy in male contraception, as has been already suggested (5).

REFERENCES

1 Duke's Phytochemical and Ethnobotanical Databases. www.rac-intl.org/publications/drduke.pdf.

2 Ajaiyeoba EO, Abalogu UI, Krebs HC, Oduola AM (1999). *In vivo* antimalarial activities of *Quassia amara* and *Quassia undulata* plant extracts in mice. J Ethnopharmacol 67: 321-325.

3 Apes S, Cimanga K, Vanden Berghe D, Van Meenen E, Longanga AO, Foriers A, et al (2002). Antiviral activity of simalikalactone D, a quassinoid from *Qassia africana*. Planta Med 68: 20-24.

4 Gilbert B, Teixeira DF, Carvalho ES, De Paula AE, Pereira JF, Ferreira JL, et al. (1999). Activities of the Pharmaceutical Technology Institute of the Oswaldo Cruz Foundation with medicinal,

insecticidal and insect repellent plants. An Acad Bras Cienc 71: 265-271.

5 Parveen S, Das S, Kundra CP, Pereira BMJ (2002). A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats. Reprod Toxicol 17: 45-50.

6 Njar VC, Alao TO, Okogun JI, Raji Y, Bolarinwa AF, Nduka EU (1995). Antifertility activity of *Quassia amara*: quassin inhibits the setroidogenesis in rat Leydig cells in vitro. Planta Med 61: 180-182.

7 Raji Y, Bolarinwa AF (1997). Antifertility activity of *Quassia amara* in male rats- in vivo study. Life Sci 61: 1067-1074.

8 Akbarsha MA, Latha PNL, Murugaian P (2000). Retention of cytoplasmic droplet by rat cauda epididymal sprmatozoa after treatment with cytotoxic agents. J Reprod Fertil 120: 385-390.

9 Agnes VF, Akbarsha MA (2003). Spermatotoxic effect of aflatoxin B(1) in the albino mouse. Food Chem Toxicol 41: 119-130.