Reproductive Impairment Associated With The Ethanolic Extract Of Alstonia Boonei (De-Wild) Stem Bark In Male Rats

G Oze, H Nwanjo, R Oze, E Akubugwo, E Orisakwe, P Aka

Citation

Abstract

The aqueous ethanolic extract of Alstonia boonei stem bark was studied on the reproductive characteristics of 60 male albino rats for 2 and 4 weeks. The rats were randomly assigned to 6 groups of 10 animal each for the 2 weeks segment: group 1 received 1ml/kg normal saline, group II and III got 50 and 200mg/kg of the extracts respectively. For the 4 weeks segment, group IV received 0.5ml/kg normal saline, while groups V and VI got 50 and 200mg/kg of the extract respectively. At the end of the 2 weeks 50mg/kg extract significantly decreased the sperm count, motility, viability and adversely charged the morphology of the sperm. These results were replicated dose and time dependently in the 200mg/kg by the 4th week. However no significant changes on the blood level of testosetorone and body weight were observed. It is concluded from this study that the extract may have toxic potentials on reproductive function without the involvement of plasma testosterone. Thus the dose and duration of application of the extract in folk medicine should be regulated to forestall possible reproductive impairment. The active constituent associated with this effect and the mechanism of action are subjects tofurther investigation.

INTRODUCTION

The application of natural medicinal products to manage the diseases of man is primordial. Man has often depended on natural substances to treat some ailments, especially in the developing economies. The trend is being reversed in recent times in some advanced countries because of the perceived long term toxicity of some synthetics drugs. Some of the natural products have provided the lead for researches, discovery, development and synthesis of many orthodox drugs in current use; for instance the 4-aminoquinolines for malaria and the cardiac glycoside, digitalis for arrhythmias.

Alstonia boonei grow wild as a lactiferous tree, and belongs to the family, Apocynaceae. It is widely distributed, but thrives best in the tropics and subtroprical regions. Studies reveal that most components of the plant have medicinal values. The phytochemical analysis shows that the plant contains alkaloids, terpenes, sterols and porphyrines. Some of them have been isolated and characterized. They include echitamine, astonine, ditanine, chlorogenine, alstovenine, etc. The pharmacological screening of the plant extract reveals the following actions. Antimalarial antipyretic (Ojewole, 1984); analgesic, antiinflamatory (Kweifio-Okai, 1996, Olajide et. al., 2000) antipsychotic, lactogenic (Yinusa et.al., 2005,) diuretic, antihypertensive (Kucera et al., 1972) anthelmintic, spasmolytic (Makinde, 1996) antidote, antimicrobial (Olajide, 2000). Studies exist to show that some antimalarial drugs possess antifertility properties. This has been documented for quinine, and chloroquine by Sairam(1978), Meisel et al (1993), and Adeeko and Dada (1998). Lohiya et al., (1994), Raji and Bolarinwa (1997) and Raji et al., (2003) have also reported the reversible antifertility activities of extracts of Carica papaya, quassia amara and Azaridichta indica respectively.

Medicinal substances emanating from extracts of Alstonia boonei are widely used in folk medicine, especially in the management of febril illness, pyrexia and malaria syndrome. This informed the study of the plant extract on reproductive function in order to generate information about the possible reproductive effect of the extract, especially in developing economy such as Nigeria where the rural population depend on the extract. The information will also be useful as a guide to folk medicine. practitioners who use the plant extract, regularly.

MATERIALS AND METHODS

PLANT MATERIAL

The stem bark of Alstonia bonnie was collected from Imo State University Owerri Campus and was identified by Dr. S. E. Okeke (plant Taxonomist) Department of Plant Biology and Biotechnology, Faculty of Science, Imo State University, Owerri. The voucher specimen is deposited in the departmental herbarium.

PREPARATION OF EXTRACT

The stem bark was cut into bits and dried in an oven at $50\,^{\circ}c$ to constant weight. It was Ground into fine powder using Thomas contact mill (Pye, Unipam England) and weighted 405g. The powder was soaked in 7.50L of 70% ethanol for 24hours and extracted with soxhlet. The extract was concentrated using Rotatory Evaporator (Laborato 4000, China). 12.40g of the ethanolic extract was obtained representing 3.06% yield of the dry powder.

ANIMAL

Male albino rats weighing 0.1-0.35kg, were obtained from the Animal House, Faculty of Agriculture University of Nigeria, Nsukka. The albino rats were housed in wire mesh cages under standard conditions of temperature and 12 hours light /dark cycle. Animals were fed with commercial diet (Agro feed, Guinea Farms, Nig. Ltd.) and water ad libitum.

EXPERIMENTAL DESIGN

The 60 male albino rats were randomly assigned to six study groups (A- F) each containing I0 rats. In addition to the normal feed and water the groups received the following drug extracts by oral tubation:

Group A, 1ml/kg of normal saline for 2 weeks (control).
Group B, 50mg/ kg Alstonia bonnie stem bark extract for 2 weeks.
Group C 200 mg/kg extract for 2 weeks
Group D 1ml/kg of normal saline for four weeks (control).
Group E, 50-mg/kg extract for four weeks.
Group F, 200 mg/kg Alstonia bonnie stem bark extract for four weeks.

BLOOD COLLECTION AND TESTOSTERONE DETERMINATION

Twenty four hours after the last doses were administered the animals were weighed and then anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean dry centrifuge tubes without anticoagulant to separate serum. The serum testosterone was estimated with Radio System Laboratory incorporated Kit, Carson, California. This method captured both protein and non- protein – bound testosterone.

COLLECTION OF SPERMATOZOA

24 hours after the last dose of respective treatments the animals were reweighed and dissected. The sperm specimens were collected by aspiration (Besley et al., 1980). This involved making an incision in the caudal of right ductus deferens and aspirating about 1.5μL of semen into a polyester capillary tube. The sample was flashed into an aliquot of 1mI of normal saline.

The sperm characteristics were determined using a Neubauer hematocytometer. Ten fields of microscope were randomly selected and sperm motility was assessed on each field (Akbarsha et el., 2001). The sperms were divided into motile, sluggish and immotile ones. The percentage of motile sperms were defined as the number of sperms divided by the total number of counted cells. The morphology of the spermatozoa were also assessed on the basis of relative comparism between normal (N) and abnormal ones (AB). The data were analysed statistically with ANOVA and Student's t-test.

RESULTS

{image:1}

Legend: The extract did not have any significant effect on the body weight of rats treated for 2 and 4 weeks.

{image:2}

There was no significant difference (P>0.05) in the serum levels of testosterone in rats treated with 50 mg/kg and 200 mg/kg of the extract for 2 and 4 weeks when compared with their control. The serum testosterone levels were 1.2 ± 0.16ng/ml in the control rats; 1.0 ± 0.23 and 1.1 ± 0.20ng/ml respectively in those treated with 50 mg/kg and 200mg/kg of the extract for 2 weeks. Also, the level was 1.46 ± 0.11ng/ml in the control rats; 1.44 ± 0.23 and 1.38 ±25ng/ml respectively in those treated with 50 mg/kg and 200mg/kg of the extract for 4 weeks. The changes in the serum level of testosterone for the two and four weeks segments were marginal and non-significant (P>0.05).

{image:3}

* Statistically different from control (P<0.05)

** Statistically different from control and within group (P<0.05)

*** Statistically different from control but less depressed than 50mg/kg

N Normal percentage morphology

AB Abnormal percentage morphology.

The sperm motility was significantly reduced (p<0.05) in rats treated for 2 weeks with 50 mg/kg. (40.6 ± 4.88) and 200mg/kg. (33.6 ± 0.61) compared with the control (82.0 ± 2.55). Similar significant reduction (P<0.05) was also observed in rats treated for 4 weeks with 50 mg/kg (32.1 ±2.17) and 200 mg/kg (26.0 ±3.10) compared with their controls (61.60 ±1.67). The reduction in motility was more in rats treated for 2 weeks with 200 mg/kg (33.6 ±0.61) compared with those treated with 50 mg/kg (40.6 ±4.88). The effect of extract at 2 weeks was found to be dose dependent (table 3). The inhibition was found to be less for 200mg/kg in the four weeks segment when compared with 50mg/kg. These seemed to be initiation of recovery of the sperm parameters by this time.

The sperm count was significantly reduced (p<0.05) in rats treated for 2 weeks with 50 mg/kg (52.2 ±2.28) and 200 mg/kg. (49.8 ±2.86). No significant reduction (p>0.05) was observed in rats treated for 4 weeks with 50 mg/kg (49.0 ± 2.65) and 200 mg/kg (51.4 ± 2.41) compared with their control (54.8 ± 1.92) (p<0.05). There was also no significant difference in the sperm count of rats treated wit 50 mg/kg for 2 weeks when compared with those treated for 2 weeks with 200 mg/kg. Similar observation were made in those treated for 4 weeks with 50mg/kg and 200 mg/kg (p>0.05).

The sperm viability was significantly reduced (P<0.05) in rats treated for 2 weeks with 50 mg/kg (50.0.3.16) and 200 mg/kg (24.2 ± 0.38) compared with the control (87.4 ±95). Similar reduction (P<0.05) was also observed in rat treated for 4 weeks with 50 mg/kg (44.6±1.67) but not with 200mg/kg (52.4 ± 3.27) compared with their control. The reduction in viability was highest in rats treated for 2 weeks with 200 mg/kg (24.2 ± 0.35) than those treated with 50 mg/kg for the same period (50 ± 3.16). The changes in the sperm viability were both duration and dose dependent (table 3) and statistically significant (p<0.05).

The abnormalities of the epididymal sperm morphology observed were too long tails, tiny and small tails, double head and biforcal tails. The degree of morphological abnormality expressed as percentage was significant (p<0.05). The morphology of rats treated with 200mg/kg for 4 weeks (72.6 ±5.27) exhibitd higher percentage abnormality than those treated with 50 mg/kg extract for 4 weeks (57.4 ±9.94). The abnormalities in morphology of the sperms were both dose and duration dependent (Table 3).

DISCUSSION

Spermatozoa posses two vital features: motility and fertility. The quantity and quality of the sperm are determined by the sperm-count, the degree of motility, viability and morphology. Some plant extracts such as Ocimum sanctum, Amaranthus spinosus, Carica papaya and Spirulina plantensis have been reported to affect spermatic qualities (Murugavel et. al., 1989, Chio & Hwang, 2005) and consequently testicular function. Drugs or chemicals that affect testicular function often affect the quantity and quality of sperm cells (Orisakwe et. al., 2003). The implication is that the administration of these extracts may cause variation in sperm count, motility, viability, morphology, and invariably affect their fertility potentials (Aladakatti et al., 2000).

The present study evaluated the reproductive impairment caused by extract of the stem bark of Alstonia boonei at two and four weeks duration. The results from the 4 weeks segment showed that there were changes in the body weights of the animals, though these were not significant (p>0.05). Changes in body or organ weights after chemical or drug administration is an indication of possible toxicity (Laumann et. al., 1995).

The serum testosterone level also decreased marginally when the test groups B,C, E and F were compared with their respective control groups A and D in the 2nd and 4th week segments. Peripheral testosterone concentration are known to affect mainly testicular secretary function (Jubiz et. al., 1974). It has also been observed that delayed spermiation impairs testosterone function (Jewel et. al.,1998) and cause decrease in sperm count. It has been reasoned that some plant extracts may cause testosterone depletion at the target sites, sparing the periphery to a large extent. This may explain why the sperm characteristics were significantly affected (P<0.05) without significant effect on serum testosterone. Moreover, androgens are essential for steriodogenesis, maturation and survival of spermatocytes (Dyson and Orgebein, 1973). Following this observation, the inhibition of serum testosterone, by the extract, though non-significant, may have a fundamental effect on the sperm

parameters.

Fifty and 200mg/kg of the plant extract in a two weeks study period significantly reduced the sperm count, percentage motility, viability and normal morphology ($p<0.05$) (Table 3), this agrees with the work of Yinusa et. al., (2005). For the four weeks study segment, the plant stem bark extract decreased the sperm parameters significantly ($p<0.05$), but to a lesser extent than the two weeks study segment. Yinusa et. al., (2005) also reported the restoration of full spermatic functions after the 12th week of study in the presence of the plant extract. The data on Table 3 showed these features except in the percentage morphology where the cyto-architecture of the spermatocytes were deranged dose and duration dependently by the 4th week. Sexana et al (1980), Raji et. al., (2003), as well as Lohiya et. al., (1994) reported similar observations. Working with niferacetam, Shinomura et. al., (2004), observed that the reduced spermatic activities began to return to normal by the 4th week of nifiracetam, administration. Nonetheless, the derangement of the sperm cell morphology found in this study may have implications for fertility and foetal defects. This can be investigated.

Some species of Alstonia such as A. Scholaris and A.spectabilis (Apocynaceae) have been shown to inhibit the release of nitric oxide (NO) in cell culture. (Choi and Hwang, 2005). Nitric oxide is known to be a potent vasodilator and smooth muscle relaxant. Its inhibition may lead to vasoconstriction, hypertrophy, ischemia, and necrosis of the reproductive cells. The result of the present study may not be unconnected with such metabolic interference in NO release. This action could have deleterious effect on the spermatocytes and reproductive characteristics of the male rats.

It is therefore concluded that A. boonei stem bark extract induced testicular damage at the tested doses and duration. The reduction in sperm count, attenuation of motility, viability and the adverse changes in the cyto- architecture of the germ cells may negatively impact on fertility in the male rats. However, there are reports where the natural agent are selectively toxic to the mammalian tissues while sparing the rodents because they posses more efficient xenobiotic biotransformation system (Laumann et. al., 1995) than man. As such, the susceptibility of man to the toxic propensities of such agents would be higher. This gives the basis for the extension of the study to human subjects, who may be the prime victims of such adverse effect; judging from the fact that A. boonei is popular as a medicinal plant especially in the rural communities of developing countries.

References

r-0. Adeeko, A. O. and Dada, O.A. (1998). Chlroquine reduces the fertilizing capacity of epididymal sperm in rats. African Journal of Med and Med. Sci., 27: 63 – 68.

r-1. Akbarsha, M.A, Kadal mani, B. Girija R., Faridha, A. and Hamid S. (2001). Spermatotoxic effect of Carbendazim. Indian J.Expt. Biol. 39: 921 – 924.

r-2. Aladakatti, R. H., Ahmed R.N. and Ghoseawar, G. (2002). Sperm parameters changes induced by Azadiracta indica root on albino rat. J. Basic and Clin physiol pharmcol, 12 (1): 3-9.

r-3. Besley, M.A., Eliarson, R., Gallegos A.J., Mognissi, K.S., Paulson, C.A., and Prassid Mr. (1980). Laboratory manual for the Examination of Human Semen and Semen cervical mucus interaction. WHO. Press. Singapore.

r-4. Bloom, W.and Facett, D.W. (1995). Male reproductive System, In: Textbook of Histology. Bloom W. Saunders Company, Philadelphia, Pp 235 – 237.

r-5. Choi, E. and Hwang, J. (2005). Screening of Indonesian medicinal plants for inhibition activity on nitric oxide production of RAW 264 .7 cells and antioxidant activity. Fitoterapi, 76:194-203.

r-6. Dyson, A.L., and Orgebein, M.C. (1973). Effect of hypophysectomy, castration, and androgen replacement upon fertilizing ability of rat epididymal spermatozoa: Endocrinology, 93: 391 – 402.

r-7. Kucera, M., Marguis, V.O. and Kucerovah, A. (1972). Contribution to the knowledge of Nigerian Medicinal Plants. TLC Separation and Quantitative Evaluation of Alstonia boonei Alkaloids. Planta Medica, 21:343-346.

r-8. Kweifio – Okai, G. (1991). Anti-inflammatory Activity of a Ghananian Antiarthritic Herbal Preparation II. J. ethnopharmacol: 33 (1-2): 129 – 133.

r-9. Kweifio – Okai, G., Bird, D., Field., F. Ambrose, R., Carrol, A.R., Smith, P. and Valdes, R. (1995). Anti-inflammatory Activity of a Ghananian Antiarrrythmic Herbal Preparation III. J. Ethnopharmacol. 46 (1):7-15

r-10. Jubiz W. (1974). Endocrinology. Atropical Approach. Jaffers MD. Amstrog T. (ed) Mc Graw Hill Kogakusha Ltd. Tokyo. Pp 235- 253.

r-11. Launmann, H., Lullmann, Warsmann, O. (1995). Rev. Tox, 4:185 – 218.

r-12. Lewis, W.H. and Elvin-Lewis, M.P.F. (1977). Medical Botany. John Wiley and Sons, Inc. , New York. Pp 200 –202.

r-13. Loyiha, N. K., Goyal, R.B., Jayaprakash, D., Ansari, A.S. and Sharma, S. (1994). Antifertility Effects of Aqueous Extract of Carica papaya seeds in Male Rats. Planta. Med., 66:400-404

r-14. Makinde, J.M. and Taiwo, O.B. (1996). Contractile Acitivity of Alstonia boonei Stem Bark on Isolated Rat Stomach Strip and Guinea Pig Illeum. Indian J. pharmacol. 28 (2): 100 –112.

r-15. Meise,. M.L., Winterhoff, H. and Jekat F.W. (1993). Tylosin inhibits the Steroidogenesis in Rat Leydig Cells in-vitro. Life Sciences 53:77 –84.

r-16. Murrugavel, T., Rukuadin, A., Thangarelu, S. Akbarsha, M. A. (1989). Anti-fertility effect of Vinca rosea (Linn) leaf extract in male mice-a sperm parameter study. Current Sci. 58: 1102 – 1103.

r-17. Ojewole, J.A.A. (1984). Studies on the pharmacology of Echitamine, an alkaloid from the stem bark of Alstonia boonei (Apocynaceae). International Journal of Crude Drug Research. 22: 121-143.

r-18. Olajide, O.A., Awe, S.O. Makinde, J.M., Ekhelar A.I., Olusola, A., Moreise, O. and Okpako, O.T. (2000). Studies on the Anti-inflammatory, Antipyretic and Analgesic Properties of Alstonia boonei Stem Bark. Journal of Ethnopharmacology, 71 (1), 179-186.

r-19. Orisakwe, O.E., Obi, E., Udemezue, O.O. and Meluda S.C. (2003). Effect of Halofantrine on Testicular Architechture & Testosterone level in gunea pigs. Eur. Bulletin of Drug. Res. 11 (4): 105. – 109.

r-20. Raji, A., Kweifio – Okai, G., Macrides, T., Sandeman, R.M; Chandler, D.S. and Polya, G.M. (2000). Inhibition of Serine Proteases by Anti-inflammatory Triterpenoids' Planta Med., 66 (3): 206 –210.

r-21. Raji, Y., and Bolarinwa, A.F. (1997). Anti fertility Activity of Quassia amara in Male Rats – in vitro study. Life Sciences. 61 (ii): 1067 – 1074.

r-22. Raji Y., Udoh, U. S., Mewoyeka, O.O., Ononye, F.C. and Bolarinwa, A.F. (2003). Implication of Reproductive Endocrine Malfunction in Male Anti-fertility Efficacy of Azaridichta indica Extract in Rats. African Journal of Medicine Med. Sci., 32: 159 – 165.

r-23. Sairam, M.R. (1978). In K.W. Mckevas (ed) Structure and Function of Gonadotrophins, pengium, New York. pp274 – 292.

r-24. Saxena, S.K., Maewal, A.K., Arora, R.C., Saxena, C.D., Gupta D., Panda DN (1980). Testosterone induced changes in rabbit serum. Indica J. Med. Res., 71: 375 – 378.

r-25. Shinomura K., Shinada, M., Hagiwara, M., Harada, S., Kato, M. and Oruhama, K. (2004). Testicular toxicity in dogs by Nifiracetam, a neurotransmission enhancer. Drug Safety Res. Laboratory. Daiichi Pharmaceutical Co. Ltd. Tokyo.

r-26. Sofowora, E.A. (1986). The State of Medicinal Plants in Nigeria. Nigerian Society of Pharmacognosy. 12 th Edition, pp. 30 – 34.

r-27. Yinusa, A.O., Mossa, J.S. and Galam, G. (2005). Phamacological studies of Alstonia boonei. International Journal of Pharmacognosy 33: 242 – 246.

Author Information

Gabriel Oze

Institute of Neuroscience and Biomedical Research, College of Medicine, Imo State University

Harrison Nwanjo

Dept of Medical Laboratory Sciences, College of Medicine, Imo University

Rita Oze

Dept of Chemistry, Federal University of Technology

Emmanuel Akubugwo

Dept of Biochemistry, Abia State University

Ebere Orisakwe

Dept of Pharmacology, Nnamdi Azikiwe Teaching Hospital Nnewi

Peter Aka

Dept. of Pharmacology/Toxicology, University of Nigeria Nsukka