https://ijrps.com

ISSN: 0975-7538
Research Article

Safety evaluations of aqueous stem bark extract of *Lophira lanceolata* in Sprague dawley rats

Etuk EU*, Muhammad AA

Department of Pharmacology, College of Health Sciences,
Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

Lophira lanceolata extract is widely used to enhance sexual performance among the male population in Sokoto state, Nigeria. The efficacy of this extract to enhance sexual behaviours in experimental animals has been reported. This study was conducted to evaluate the safety of oral administration of the plant extract in Sprague dawley rats. Various concentrations (500, 1000, 3000 and 5000mg/kg body weight of aqueous stem bark extract of *Lophira lanceolata* were administered orally to four groups of rats to test for acute toxicity. Also, the effects of oral administration of the extract at 1000, 1500 and 2000 mg/kg body weight for 28 days on the body weight, some haematological and biochemical parameters of the animals were investigated. The result showed that, a single oral dose treatment with up to 5000mg/kg of the extract did not cause any dead or observable adverse effect in the rats. In the repeated dose study, the extract produced an increase in the body weight gain of the rats. The total white blood cell count and haemoglobin level were elevated. These were considered to be beneficial and indicative of safety. The plant extract at 1500 and 2000mg/kg body weight produced a slight increase in the serum transaminases enzymes. The histology of the liver and kidney were normal while the testes showed some pathological lesions. These results have shown that, repeated administration of appropriate dose of the extract may have some beneficial effects on the blood system but a high dose may damage the testes thereby causing infertility. Consumers should therefore be aware of these health risks and avoid over consumption of the extract.

Keywords: Sexual stimulant, toxicity, parameters, *Lophira lanceolata*.

INTRODUCTION

Lophira lanceolata van tiegh ex.keay commonly known as iron wood belongs to the family, Ohanacea, distributed in West and Central Africa including the Northern States of Nigeria. The vernacular names of the plant in Nigeria include; Namijin Kade in Hausa, Ikponhon in Yoruba, Okopia in Igbo, and Maganchi in Nupe. The plant is native to Africa and it is found mostly in Sudan, Uganda, Benin and Cote de-Voire (Abdullahi et al., 2003). It is a shrub of up to 5 meters tall and sometimes grows up to the size of a big tree with short branches. The stem bark of the plant was reported to be grayish, rough and broken into thin corky patches (Abdullahi et al., 2003). It is usually found as a regrowth due to bush fire in the savannah. The leaves are narrowly elongated, rounded at the apex, shiny grayish to pink when young and shiny green when mature. The leaves are very prominent in appearance. The flowers occur from October to January, fruits are 0.1cm in diameter with one elongated seed. Fruiting period is between February to April. The stem bark extract of the plant has been reported to contain tannins, alkaloids, resin, saponin and flavonoid (Gill, 1992).

A study conducted by Pengyeub et al. (1994) on the stem bark extract of *Lophira lanceolata* showed different types of flavonoids, some of which posesses antibacterial and antiviral activity. The research was extended to the leaves of this plant from which two new minor bioflavonoid constituents, *Lanceolata* A and B were isolated and characterized. *Lophira lanceolata* has received considerable attention from scientists as a potential source of drug.

The issue of diminishing sexual performances among the male population though may be associated with aging process has always been a matter of great concern to man. Studies have shown that, despite the high prevalence and psychological consequences of the disorder, relatively few men had sought for orthodox treatment prior to the introduction of sildenafil (Mackinley et al., 1999). Most suffers do resort to locally available remedies. The use of sexual stimulants has a long and fascinating history (Nickel, 1999). Some of the most ancient plant based aphrodisiacs such as ginseng and yohimbine are as popular today as in ancient times. A number of rural physicians and fertility ex-

* Corresponding Author
Email: etuk2005@yahoo.co.uk
Contact: +2348054693770 Fax: +23460231514
Received on: 19-11-2009
Revised on: 22-12-2009
Accepted on: 26-12-2009

perts have discovered a combination of exotic herbs from the Amazon jungle, India, China, Malaysia and Africa that promote female and male sexual activity (Sahelian, 2004). The use of all these substances to improve sexual performances especially among the males is not without adverse effects.

Lophira lanceolata stem bark extract is very popular among the male population in Sokoto state because of its sexual enhancement properties which has been confirmed through our previous investigations (Etuk et al., 2009). But beyond the efficacy of herbal remedies, there is always a serious concern for the safety. The present study examined the safety of *Lophira laceolata* stem bark extract following oral administration in male Sprague dawley rats.

MATERIALS AND METHODS

Collection and identification of the plant

The plant *Lophira lanceolata* was identified and collected in June 2008 from its natural habitat at Fakon Idi area in Sokoto North Local Government Area of Sokoto State, Nigeria with the assistance of an Herbal practitioner. It was authenticated by a Botanist in the Biological Sciences Department, Usmanu Danfodiyo University Sokoto (UDUS). A specimen of the sample collected was labeled (PS-09-011) and deposited at the herbarium of Department of Biological Sciences, UDUS for future reference.

Preparation of plant material and extract

The stem bark of the plant was cleaned and air dried in a container to constant weight. The dried material was pounded with a mortar and pestle to form a dry powder. The extraction was carried out by weighing 250g of the powder plant material into 3.75 litres of distilled water in a conical flask. The mixture was allowed to stand for 6 hours with only intermittent shaking of the container. Filteration was done using Whitman filter paper (Odebiyi and Sofowora, 1979). The water in the filtrate was evaporated by drying in an oven at a temperature of 45^{o}C. The percentage yield was calculated from the weight of the extract residue and that of the initial material. The extract was then stored in a Deep Freezer at -15ºC and the required concentrations were reconstituted when needed for the experiments.

Experimental animals

Adult male Sprague dawley rats weighing between (186-200) g were obtained from the experimental animal unit of the Faculty of Veterinary Medicine of Ahmadu Bello University, Zaria and used for this study. The animals were housed in cages and maintained in the animal Facility of the Department of Pharmacology, Usmanu Danfiodiyo University Sokoto for 14 days before the commencement of the drug treatment. They were given free access to rat feed (Nemeith livestock feeds Nigeria Ltd) and tap water under normal labouratory environmental conditions. The study protocol was approved by the Institutional Ethical Committee (Ref. No.: UDU/DEC/09/Vol.1) for use of animals.

Acute toxicity test

The single dose toxicity test was conducted according to the Lorke method (1983). Thirty five adult male Sprague dawley rats weighing between (186-200)g were used. They were randomly divided into five equal groups of seven rats each, labeled A to E. Various concentrations (500, 1000, 3000 and 5000mg/2ml) of the crude extract were prepared. The rats in groups A to D were treated orally via a stomach tube size 35 with 500, 1000, 2000 and 5000mg/kg body weight of the extract respectively, while the rats in group E were given distilled water to serve as control. The animals in each group were constantly observed for the initial first hour for any sign of behavioral, somatic changes or death. Then intermittently for the next 6 hours, and subsequently after 24, 48 and 72 hours. The observations were recorded.

Repeated doses toxicity assessment

Twenty eight adult male rats weighing between (186-200)g were used for this study. The animals were weighed individually and1000, 1500 and 2000 mg/kg body weight of the extract were administered orally through a stomach tube size 35 to three groups of rats (n=7) labeled A-C respectively for 28 days. The fourth group which serves as control received equivalent volume of distilled water. The animals were fed and given free access to water and their body weight measured on the last day. The animals were anaesthetized on the 29th day with chloroform. Blood samples were collected for haematological and biochemical analysis through cardiac puncture. The animals were dissected, the liver, kidney and testis removed and weighed individually. Tissue samples were collected and preserved in 10% Formalin for histological examination.

Statistical analysis

The data obtained were analyzed using One-way Analysis of Variance (ANOVA) and the comparison between the control and experimental groups was done using the Turkey-Kramer's test. The level of significance was set at $P< 0.05$. The data were expressed as Mean $\pm$ Standard Deviation.

RESULTS

The extraction of *Lophira lanceolata* stem bark with water produced a dark brown colour residue which was tasteless with no peculiar smell and the percentage yield was found to be 34.2% w/w. The oral administration of 500, 1000, 3000 and 5000 mg/kg body weight of the extract did not produce any mortality or obvious signs of adverse effect in the animals during the period of observation. In the repeated dose study, administration of the extract for 28 days at concentrations of 1000, 1500 and 2000mg/kg body weight did

Table 1: Body weight of rats treated with various concentrations of *Lophira lanceolata* extract for 28 days.

Treatment group	Initial body weight (g)	Final body weight (g) (mg/kg)	% increase in body weight (%)
Control	188.0 ± 8.4	188.0 ± 8.4	1.6
1000	182.0 ±12.5	182.0 ±12.5	4.7
1500	259.7 ± 12.5	259.7 ± 12.5	3.3
2000	244.0± 14.6	244.0± 14.6	3.7

n = 7; Values = Mean ± SD.

increase the percentage body weight gain of the animals (Table 1).

Also, the extract produced a significant increase ($p<0.05$) in white blood cell count and a non significant increase ($p>0.05$) in the haemoglobin levels of the animals (Table 2). The extract did not have any effect on the urea, glucose, cholesterol and total serum protein values in the treated animals when compared to the control group. However, the levels of the liver enzymes, Alanine aminotransferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) in all the treated groups were slightly increased when compared to the control (Table 3)

The histological examination of the liver and kidney revealed no abnormality in the architecture of the organs (Figures 1a & b). But there was mild hyperplasia of the Leydig's cells, and Giant cells infiltration in the testes of the rats treated with 1500 and 2000mg/kg body weight of the extract. Also some areas of focal necrosis, calcification, oedema and thickening of the basement membrane were visible (Fgures 2a, b, c & d).

DISCUSSION

This study was conducted to establish how *Lophira lancoelata* stem bark extract; a popular aphrodisiac agent in Sokoto State can be used safely. Previous studies have shown that, there is no safe drug but rather safe dose of a drug (Mann et al., 1997). As Paul Bergner, pointed out: Approximately 8% of all hospital admissions in the United States are due to adverse reactions to synthetic drugs (Ramsey, 2000). Toxicity of herbal medicines needs to be seen in this context.

The results have shown that, a single oral dose administration of up to 5000mg/kg body weight of the extract did not produce any death or visible adverse effect in the animals. This indicates that, the extract is relatively safe. Several studies of medicinal plants in Nigeria and Africa have reported the lethal median dose of 2000mg/kg body weight and above to be safe following oral administration in rats and mice (Abatan and Arowolo, 1989; Etuk et al., 2005).

Also administration of the extract for 28days increased the percentage body weight gain in the rats when compared with the control group. This finding is very

Table 2: Effect of daily administration of extract for 28 days on haematological parameters in rats

TREATMENT GROUP mg/kg	RBC $x10^6$/l	MCV (fl)	PCV (%)	PLT 10^5/ul	WBC 10^3/ul	HGB (g/dl)	MCHC (g/dl)
CONTROL	7.7±0.3	63.4±3.0	48.3±3	429±90	6.1±3.8	12.5±0.5	26.0±2.3
1000	5.5±1.2	63.9±2.6	35.4±15	182±39	12.8±0.5*	12.8±1.3	28.1±3.5
1500	7.7±0.7	65.3±4.2	50.1±6.5	335±15	11.2±3.5*	13.5±0.2	27.0±1.4
2000	7.5±0.8	65.4±2.6	48.1±7.7	4571±9	11.7±2.6*	13.8±1.2	27.7±0.9

Values= Mean ± SD, n = 7, RBC= Red blood cell, MCV= Mean corposcular volume, PCV = Pack cell volume, WBC= White blood cell, HGB= Haemoglobin, MCHC= Mean corposcular haemoglobin concentration,
* = Significant change when compared with control, $p<0.05$.

Table 3: Effect of daily administration of extract for 28 days on biochemical parameters in rats

Parameters	Treatment groups(mg/kg)			
	Control	1000	1500	2000
Urea(mmol/l)	3.78 ±0.18	2.96±1.03	3 .17±0.15	3.63±0.72
RBS (mmol/l)	5.16± 0.12	5.28± 1.1	4.98±1.1	5.13±0.8
Total Cholesterol (mmol/l	67.07±1.2	65.91± 2.1	67.33±0.7	63.40±1.9
Total serum protein(g/l)	7.30±1.2	8.11±0.9	8.01±0.1	7.41±0.7
AST(IU/l)	36.0±2.4	41.2±1.1	41.7±3.4	39.8±4.6
ALT(IU/l)	53.2±0.5	59.0±2.3	61.2±5.1	58.3±0.9
ALP(IU/l)	24.9±3.9	35.0±0.1	28.2±0.6	37.5±2.2

n = 7; Values= Mean ± SD, RBS = Random blood sugar, AST = Aspartate aminotransferase , ALT = Alanine aminotransferase, l = Litre, g = gram, mmol = millimole, ALP = Alkaline phosphatase, IU = International Unit, $p<0.05$.

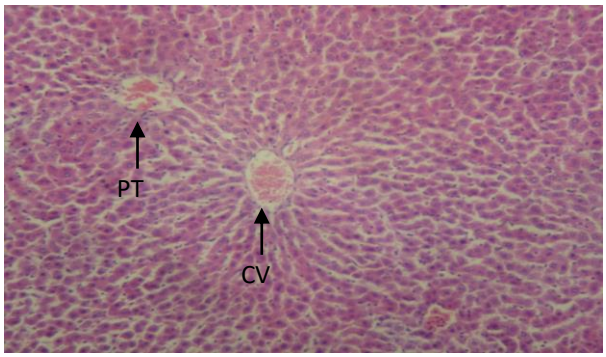

Figure 1a: Photomicrograph of the rat liver treated with 2000mg/kg of extract showing normal architecture PT= Portal tract; CV = Central venule (x 10)

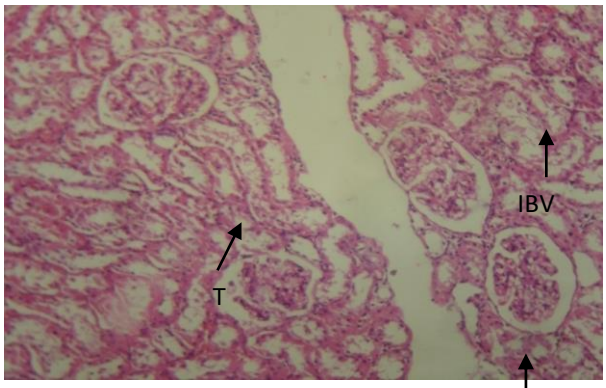

Figure 1b: Photomicrograph of rat kidney showing normal features after 28days treatment with 2000mg/kg of the extract. [T = Tubules; GM = Glomeruli; IBV= Interstitial blood vessels (x 10)]

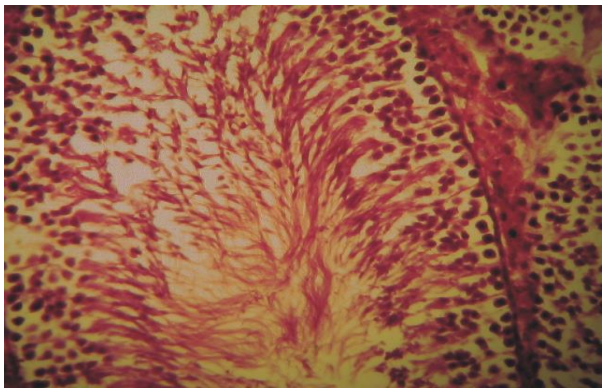

Figure 2a: Photomicragh of rat testes showing spermatogenesis in control group (Carbol fuschin stain) (x 40)

important because, the toxicity of chemical compounds in experimental animals is often associated with loss of body weight (Parveen et al., 2003; Etuk et al., 2009). A previous study has shown that, the administration of the extract produced an increased in the serum levels of testosterone in treated rats (Etuk & Muhammed, 2009). The increase in the body weight of the rats as recorded in this study may be due to the ability of the extract to increase the levels of this steroid hormone (androgenic compound) in the animals. Androgenic compounds possess anabolic activity (Marin et al., 1992).

From the results of the haematological analysis it was observed that, the extract of *Lophira lanceolata* at

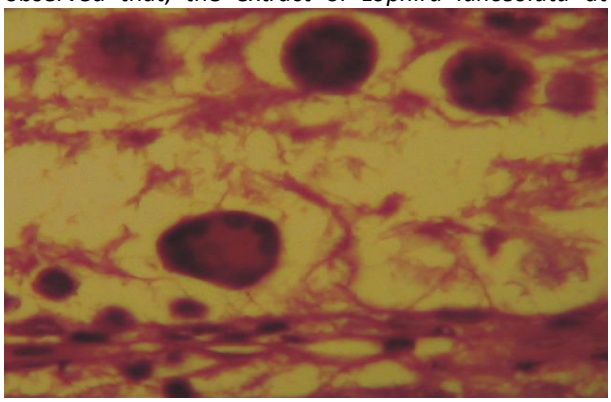

Figure 2b: Photomicrograph of rat testes showing giant cells after administration of 1500mg/Kg of extract (Carbon fuschin stain) (x40)

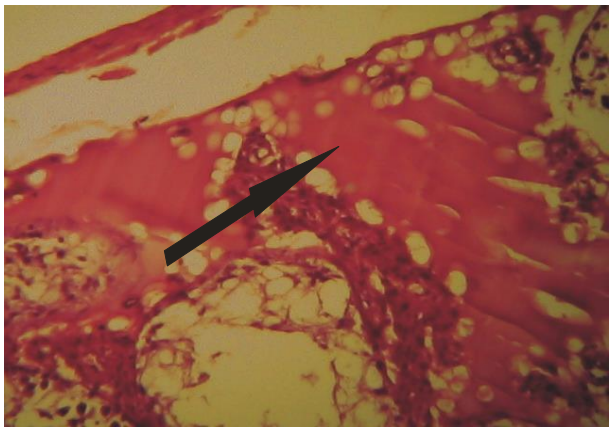

Figure 2c: Photomicrograph of rat testis showing oedema in the interstitium after administration of 2000mg/Kg of the extract (Carbon fuschin stain) (x40)

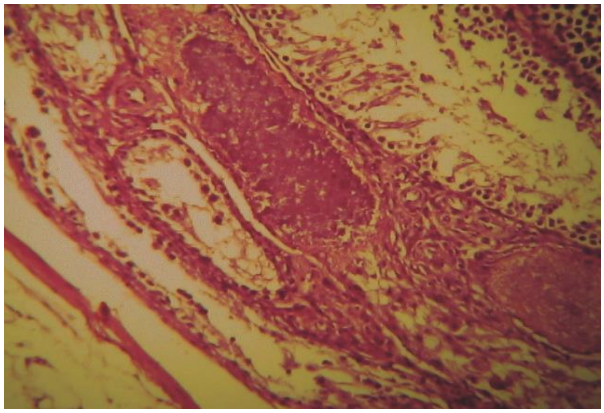

Figure 2d: Photomicrograph of rat testis showing dystrophic calcification and necrosis after the administration of 2000mg/Kg of the extract (Carbon fuschin stain) (x40)

different doses increased the haemoglobin level and the white blood cell count in the rats. Blood is an index of physiological and pathological status in animals and the parameters usually measured are haemoglobin, packed cell volume, white blood cell count, platelets count (Schalms et al., 1975). The values of these pa-

rameters can be changed by the ingestion of some toxic plants (Abatan and Arowolo 1989., Ajagbonna et al., 1999). But in this case, an increase was observed which may indicate the beneficial effect of the plant on the blood system. The increase in these parameters may be as a result of the direct effect of the extract on haemopoitic sites in the animals or the effect of the androgenic hormone.

Repeated treatment with the extract produced a slight increase in the values of the liver transaminases enzymes. Serum ALT is known to increase when there is liver cells damage and it has been used as a tool for measuring hepatic necrosis (Bush, 1991). AST however, is not a liver specific enzyme as high levels of the enzyme can also be found in skeletal and cardiac muscles as well as red blood cells (Adedapo et al., 2007a). Increase in serum ALP may be considered as an indicator of cholestatis which may result from intracellular hepatic canaliculli obstruction associated with inflammation. The absence of pathological lesions in the liver to support these biochemical changes may be due to the mild toxic effect of the plant or the short duration of treatment. However, the severe damage produced on the testes (Figures 2b, c & d) by the extract may be as a result of over stimulation which may cause tissue hypoxia and cell dead. There has been a previous report of testicular toxicity in male wistar rats produced by a commercial herbal medicine (Etuk & Egua, 2009).

CONCLUSION

The oral administration of *Lophira lanceolata* stem bark extract may be relatively safe as shown from the results of this study. The extract administration is associated with increase body weight, improvement in some haematological parameters and no acute toxic effect, but repeated administration of high doses of the extract may result in testicular damage thereby causing infertility. Users of this extract should avoid excessive consumption.

REFERENCES

Abatan MO, Arowolo RO. Toxicity of *Eugenia uniflora* to rats. N. J. An. Product. 1989, 16: 16-19.

Abdullahi M, Muhammed M, Abdulkadir NU. Medicinal and Economic Plants of Nupeland,1st edition, Jube Evans books and publications, Bida, Nigeria, 2003, pp. 276.

Adedapo AA, Abatan MO, Olorunsogo OO. Effects of some plants of the spurge family on the haematological and biochemical parameters of rats. Vet. Arhiv. 2007a, 77: 29-38.

Ajagbonna OP, Onifade KI, Suleiman U. Haematological and Biochemical changes in rats given extract of *Calotropis procera*. Sokoto J. Vet. Sci. 1999, 3 (2) : 30-34.

Bush BM. Interpretation of laboratory results for small animal clinicians. Blackwell Scientific Publications, London, 1991, pp. 56-59.

Etuk EU, Adebiyi R, Elsa AT, Agaie MA. Acute and sub-chronic toxicity studies of aqueous root extract of *Securidaca longepedunculata* in mice. Int. J. Pharmacol. 2005, 2(4): 421- 424

Etuk EU, Egua MO. Testicular toxicity of a commercial polyherbal product in male albino rats. Nig. J. Basic and Appl. Sci. 2009, 17: 19 - 26

Etuk EU, Igbokwe V, Ajabonna OP, Egua MO Toxicological studies of a Nigerian polyherbal product. Res. J. Med. Plants 2009, 3(2) 52-60.

Etuk EU, Muhammed AA. Fertility enhancing effects of aqueous stem bark extract of *Lophira lanceolata* in male Spargue dawley rats. Int. J. Plant Phys. Biohem. 2009, 1(1): 001-004.

Etuk EU, Muhammed AA, Igbokwe V, Okolo RU. Sexual stimulatory effects of aqueous stem bark extract of *Lophira laceolata* in male *Sprague dawley* rats. J. Clin. Med. Res. 2009, 1(2) 18-21.

Gills LS. Ethnomedical uses of plants in Nigeria. Uniben Press, Edo state, Nigeria 1992, pp. 121.

Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983, 54: 275-287.

Mann H, Ladenheim B, Hirata H, Moran TH, Lud Cadet J. Differential toxic effects of methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) in multidrug-resistant (mdr1a) knockout mice. Brain Research 1997, 769(2):340-346

Marin P, Holmang S, Johnson L, Sjostrom L, Kvist H, Holm G, Linstedt G, Bjorntorp P. The effect of testosterone treatment on body composition and metabolism in middle age obese men. Internetional Journal of Obesity 1992, 16(12): 991-992.

Mckinley J, Aytac IA, Krane RJ. The likely Worldwide increase in Erectile Dysfunction Between 1995-2025 and some possible consequences. B. J. Urol. Int. 1999, 84 (10) 50-56.

Nickel NL, Morales A. Erectile dysfuncton in the aging man. B. J. Urol. Int. 1999, 83: 1267-1278.

Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian Medical Plants – part 1. 2nd OAU/STRC. Inter-Africa Symposium on Trade Pharmacopeia and African Medicine Plants. Pub. 1979, 115-216.

Parveen S, Suwagmani D, Chandra PK, Perreira BMJ. A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats. Repro. Toxicol. 2003, 17(1): 45-50.

Pengyeub DE, Ghogomu-Tih R, Sondemgam BL. Minor Biflavonoids of *Lophira lanceolata* J. Nat. Prod. 9 1994, (57): 1275-1278.

Ramsey L. Fertility enhancing herbs. http://www. suite101.com/print_article.cfm/preconception/4721 2 2000, 1-6.

Sahelian D. Natural Sex Boosters, supplements that enhance stamina, sensation sexuality for men and women. Wikepedia, the free encyclopedia. 2004, ISBN O-312- 19584-2.

Schalms OW, Jain NC, Caroll EJ. Veterinary Haematology, 3rd Edition, Lea and Tebiger Publishers, Philadelphia 1975, pp 207-209.