

## Original Article

## Life Science

# *Laurus nobilis* Extract Preserves Testicular Functions in Cryptorchid Rat

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## ABSTRACT [ENGLISH/ANGLAIS]

Bay leaves (*Laurus nobilis*) are spices used in cooking due to their flavoring capacity and aroma. Bay leaves are traditionally used in treating symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence. This study was aimed at evaluating the ability of polyphenolic and antioxidant-rich bay leaf extract (BLE) to protect testicular malfunction in experimental cryptorchidism based on histopathological and biochemical clarifications. Forty male wistar rats were divided into four groups of ten animals each. The first group served as the control, the second and the fourth group received 60 mg/kg body weight of BLE daily for fifty six days. The third and fourth group was rendered cryptorchid with the fourth group subsequently treated orally with 60 mg/kg body weight of BLE daily for fifty six days. The animals were sacrificed and testis weight/volume and sperm parameters were determined. Animals with untreated cryptorchidism showed significantly reduction in testis weight/volume ( $p < 0.05$ ), testis weight/body weight ratio, sperm parameters ( $p < 0.005$ ) compared to the control and group treated with BLE-alone. Treatment of the cryptorchid rats with BLE significantly improved the sperm parameters ( $p < 0.05$ ) and testicular SOD and CAT activity levels when compared to cryptorchid rats that were not treated. This showed that deleterious and degenerative changes associated with cryptorchidism were mildly averted by simultaneous treatment with BLE.

**Keywords:** Cryptorchidism, bay leaf, testis, wistar rat, histopathology

## RÉSUMÉ [FRANÇAIS/FRENCH]

Bay feuilles (*Laurus nobilis*) sont des épices utilisées dans la cuisson en raison de leur capacité arôme et arôme. Feuilles de laurier sont traditionnellement utilisés dans le traitement des symptômes de problèmes gastro-intestinaux tels que ballonnements épigastriques, mauvaise digestion, éructation, et des flatulences. Cette étude visait à évaluer la capacité des polyphénolique et riche en antioxydants feuille de laurier extrait (BLE) pour protéger un dysfonctionnement testiculaire chez cryptorchidie expérimentale basée sur des éclaircissements histopathologiques et biochimiques. Quarante rats mâles Wistar ont été divisés en quatre groupes de dix animaux chacun. Le premier groupe a servi de témoin, le deuxième et le quatrième groupe a reçu 60 poids corporel mg / kg de BLE par jour pendant cinquante-six jours. Le troisième et quatrième groupes a été rendue cryptorchidie avec le quatrième groupe par la suite traités par voie orale avec 60 poids corporel mg / kg de BLE par jour pendant cinquante-six jours. Les animaux ont été sacrifiés et les testicules paramètres de poids / volume et le sperme ont été déterminés. Les animaux atteints de cryptorchidie non traitée a montré de façon significative la réduction du poids des testicules / volume ( $p < 0,05$ ), du poids des testicules / body rapport au poids, les paramètres spermatiques ( $p < 0,005$ ) par rapport au témoin et le groupe traité avec BLE-seul. Traitement des rats cryptorchides avec BLE considérablement amélioré les paramètres spermatiques ( $p < 0,05$ ) et SOD des testicules et les niveaux d'activité CAT par rapport à des rats cryptorchides qui n'ont pas été traités. Cela montre que des changements délétères et dégénératives associées à la cryptorchidie ont été légèrement écarté par un traitement simultanément avec BLE.

**Mots-clés:** La cryptorchidie, la feuille de laurier, du testicule, rat Wistar, l'histopathologie

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## INTRODUCTION

For spermatogenesis to occur properly, a temperature few degrees lower than the body temperature has to be maintained [1]. This desired temperature is attained by testicular descent into the scrotal sac. Cryptorchidism, the failure of the testes to descend, exposes testis to higher body temperature which results in disruption of

spermatogenesis [2]. Considerable morphological and degenerative changes have been observed in both unprompted and experimental cryptorchidism [3-7]. .. Although Hall *et al.* [3] reported that steriodogenic functions of the somatic cells (Leydig and Sertoli cells) appear to be normal when exposed to the core body temperature [8] the elevated testicular temperature still

affects their normal functions to some extent [9]. Wu and Murona [10] showed that cryptorchidism causes Leydig cell hypertrophy and hyperplasia along with decreased steroidogenesis via the activation of some mitogenic factors that released from Sertoli cells [11]. Testicular maldescend is a common urological disorder normally detected at birth or shortly after birth [12]. It was thought that cryptorchidism occurs in childhood only due to either testicular dysgenesis or malformation of the inguinal tract or insufficient gonadotrophin secretion [13], but recently it has been reported that cryptorchidism has many causes including genetic, epigenetic, and environmental components [12]. In infants, the occurrence is 3.4% to 5.8% and, 0.8%- 1.8% of males remain cryptorchid even after attaining maturity [14, 15]. Post pubertal abnormalities associated with cryptorchidism are not caused by increased temperature of an abdominal location, but as a delayed manifestation of testis dysgenesis [16]. Such core testis dysgenesis of fetal origin might be evident in males who are not cryptorchid [17-19].

Molecular and cellular mechanisms of these abnormalities associated with cryptorchidism are not well known but comprehensive insults such as increased oxidative stress [20], loss of germ cell-specific glucose transporters [1, 21], and differential response to gonadotropins may contribute to gonadal cell failure [3, 22]. The physiological significance of these changes has been reported in some cases but their exact role in testicular degeneration is mostly unclear.

Therapeutic management of cryptorchidism has been limited to orchidopexy, hormonal treatment and surgical corrections [4, 23-25]. In 1997, Lenzi et al [26] showed that in humans, prepubertal orchidopexy restored sperm counts and motility parameters better than post pubertal surgical correction [26].

Oxidative damage plays an important pathological role in cryptorchidism and other human conditions [26, 27]. Several diseases have been associated with free radical generation and subsequent oxidative damage [27, 28-31]. Excess generation of reactive oxygen species induced by various processes in an organism exceeds the antioxidant capacity of the organism, leading to a variety of pathophysiological conditions [32, 33]. In recent times, antioxidants from plant materials have been helpful in the fight against free radicals generation [28, 30]. Plants like bay leaf (*Laurus nobilis*) have been shown to possess antioxidants [34], insecticidal properties [35], antibacterial, antifungal, hypoglycaemic in alloxan-induced diabetes

[35], anticarcinogenic [36], antiinflammatory effects [37], and antiulcerogenic properties [38, 39]. Traditionally, Bay leaf extract (BLE) have been used orally to treat gastrointestinal problems, epilepsy, neuralgia and flatulence [40-42].

The active components of bay leaves extract can be represented by essential oils, isoquinoline alkaloids, vitamin E, lauric acid, parthenolides, cuminaldehyde, dimethylstyrene, eugenol, methyl eugenol, carvacrol, monocyclic 1,8-cineole, alpha-terpinyl acetate, terpinene-4-ol, bicyclic monoterpenes such as alpha and beta-pinene, sabinene, and the acyclic monoterpenes such as linalool, myrcenol, and sesquiterpenes [43-47].

In 2010, Abdulrahim [46] reported that Bay leaves extract (BLE) reduces total cholesterol, low density lipoprotein, triglycerides and glucose, and increased high density lipoprotein levels in people with type 1 diabetes [46].

In a study done by Lakshmi et al [48] significant increase in the levels of lipids and lipid peroxidation products and a decline in antioxidant potential were observed in diabetic rat brain synaptosomes [48]. The scavenging activity of bay leaf is attributed to the presence of antioxidant phytochemicals [34, 48]. The purpose of this present study is to evaluate the protective efficacy of bay leaf extract (BLE) on toxicity associated with experimental cryptorchidism in rat.

## MATERIALS AND METHODS

### Plant Materials and the Aqueous Extraction Procedure

Samples of leaves were bought from a local market in Lagos, Nigeria in the month of April, 2011. They were authenticated by a staff in the herbarium of the Department of Botany, University of Lagos, Nigeria, where a voucher specimen was deposited for reference with specimen number DSN66. Bay leaves were dried and the aqueous extraction process was done as described by Elmastaş et al. [30]. The extract was stored in bottles at -20°C until use.

### Animals

Wistar rats were obtained from a breeding stock maintained in the Animal House of the College of Health Sciences, University of Ilorin, Kwara state, Nigeria and were housed in well ventilated wire wooden cages in the Animal Facility of the Department of Anatomy, Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos. The rats were maintained under standard natural

photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e. L: D; 12h:12h photoperiod) at room temperature. They were allowed unrestricted access to water and rat chow. They were allowed to acclimatize for 2 weeks before the commencement of the experiments. The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d= 0.0001 g) (Satorius GA, Goettingen, Germany). Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (49). Further the animal experimental models used 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 [50].

### Acute Oral Toxicity Study

As described earlier by Akunna et al [32], the acute oral toxicity study of BLE was conducted using the Organization for Economic Cooperation and Development (OECD) (2000) Guidance Document on Humane End points that should reduce the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure. Briefly, 5 animals were weighed and individually identified. The first animal was given the test dose -BLE 2000 mg per kg body weight. The second and third animals were concurrently dosed and the fourth and fifth animals sequentially dosed. The results were evaluated as follows (S = Survival, X = death). The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a total period of 14 days. All observations were systematically recorded with individual records maintained for each animal.

### Animal Grouping and Experimental Protocol

Forty male adult wistar rats weighing 200-250 g were used for the study. The rats were randomly divided into four groups of twelve rats each. Group A served as the

control and the rats were neither treated nor rendered cryptorchid. Group B rats were treated alone with BLE. Rats in group C were only rendered bilaterally cryptorchid while rats in group D were rendered bilaterally cryptorchid and also treated orally with 60 mg/kg body weight of BLE daily for fifty six days [26, 35]. Procedure to induce cryptorchidism was performed as described by Saalu et al [35].

### Animal Sacrifice and Sample Collection

The rats were weighed and then anaesthetized just before they were sacrificed. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Then the testes were excised and trimmed of all fat. The testes weights of each animal were evaluated. The testes were weighed with an electronic analytical and precision balance (BA 210S, d=0.0001-Sartorius GA, Goettingen, Germany). The testes volumes were measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation. One of the testes of each animal was fixed in 10% formal-saline for histological examination.

### Determination of Sperm Characteristics

As described by Akunna et al [32], the testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues. From each separated epididymis, the cauda part was removed and placed in a beaker containing 1 mL physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to release its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined as earlier described [35]. Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

### Estimation of plasma levels of testosterone

As earlier described Akunna et al [32], Plasma testosterone concentrations were estimated using the Enzyme Immunology Assay (EIA). Plasma samples were collected and stored at -20°C until assayed. The EIA kits used were obtained from Immunometrics (London U. K)

and contained testosterone EIA substrate reagents and EIA quality control samples. A quality control sample was run for the hormone at the beginning and at the end of the assay variation. The EIA kit used had a sensitivity level of 0.3 nmol/L (0.1 ng/mL). The intra and inter assay variations were 11.00 and 10.10%, respectively.

### Assay of testicular enzymatic antioxidants

#### Assay of catalase (CAT) activity

Catalase activity was measured according to the method of Aebi [51] as described by Akunna et al [32]. 0.1 ml of the testicular homogenate (supernatant) was pipetted into cuvette containing 1.9 ml of 50mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The rate of decomposition of H<sub>2</sub>O<sub>2</sub> was measured spectrophotometrically from changes in absorbance at 240nm. Activity of enzyme was expressed as units /mg protein.

#### Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured according to the method of Winterbourn [52] as described by Saalu et al [34]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). Briefly, the reaction mixture contained 2.7 ml of 0.067M phosphate buffer, pH 7.8, 0.05 ml of 0.12mM riboflavin, 0.1 ml of 1.5mM NBT, 0.05 ml of 0.01M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10 minutes. Control without the enzyme source was included. The absorbance was measured at 560nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. It was expressed as u/mg protein.

#### Histological analysis

As described by Saalu et al [34], the organs were cut in slabs of about 0.5 cm thick and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Serial sections of 5 µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal

concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven-dried. Light microscopy was used for the evaluations.

#### Statistical Analysis

The data were expressed as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). Statistical significance between the various groups was separated by t – test (SAS, 2002).

#### Acute oral toxicity

There were no deaths of mice dosed at 2000mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD50 was calculated to be greater than 3000mg/kg body weight /orally.

**Table 1:** This table shows the results of Acute Toxicity Test for BLE (Up and Down Procedure) in Rats

Test serial number	Animal Identity	Dose of MoE mg/kg	Short term result(48hrs)	Long term results (14days)
1	REP	2000	S	S
2	LEP	2000	S	S
3	TC	2000	S	S
4	RLT	2000	S	S
5	LLT	2000	S	S
6	I	2000	S	S

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RLT = Right leg tagged; LLT=Left Leg tagged, I= Intact rat

#### Body weight changes

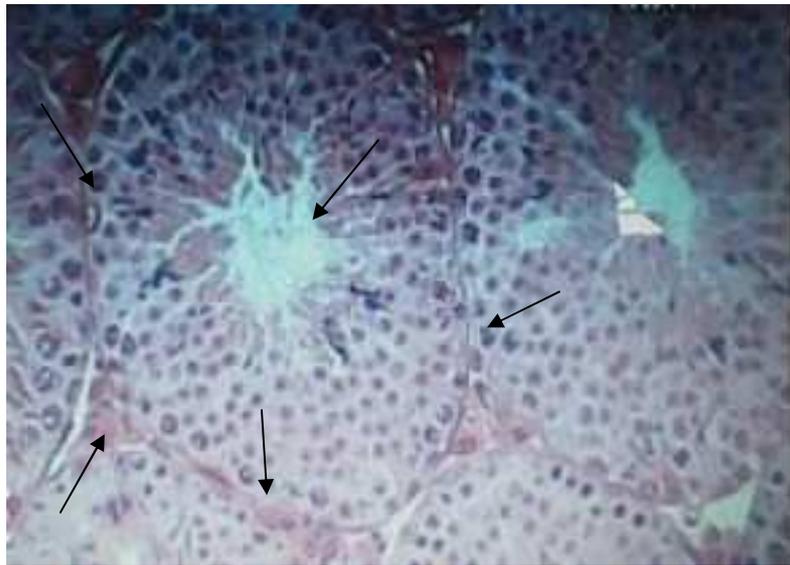
Table 2 show that animals in control group increase in weight significantly. Both cryptorchid groups lost weights when compared with their initial weights. However the weight loss by the cryptorchid rats treated with BLE was higher than the losses by cryptorchid group that did not receive BLE.

#### Weights and Volume of testes mean

Table 2 showed that the testicular weights, testis weight/body weight ratio and volumes of the cryptorchid rat were the least, being significantly ( $p<0.05$ ) lower when compared to the mean testicular weights, testis weight/body weight ratio and volumes of the cryptorchid rats exposed to BLE.

**Table 2:** This table shows the gross anatomical parameters

Anatmical Parameters	Control	BLE-alone	Cryptochid	Cryptochid/BLE
Initial Body weight	205.03±4.5	220±5.3	240.6±5.5	258±6.1
Final Body weight	235.12±3.2	234.3±2.1	235.2±3.4	225.33±2.1
Bodyweight difference	30.09	14.3	5.4*	33
Testicular weight	1.63±0.3	1.40±2.1	0.61±2.2*	1.18±3.4
Testicular Volume	1.60±2.4	1.47±5.1	0.57±2.1*	1.11±0.23
Testis w.t/B.w ratio	0.008	0.006	0.003	0.005

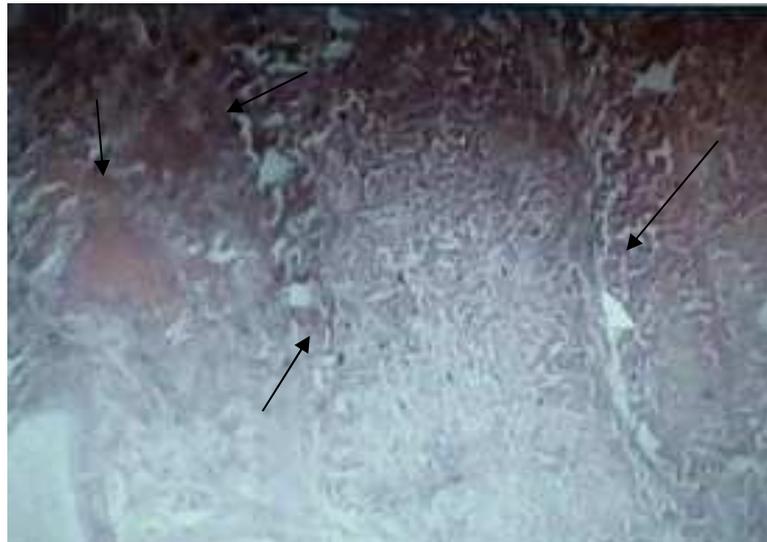
\* $p < 0.05$  compared to control group (n=10)**Figure 1:** This figure shows the normal testis with normal epithelium and intact interstitium.

(Haematoxylin and eosin stains; X 400)

**Figure 2:** This figure shows the testis of rat treated with BLE with normal seminiferous tubules and numerous spermatozoa in the lumen

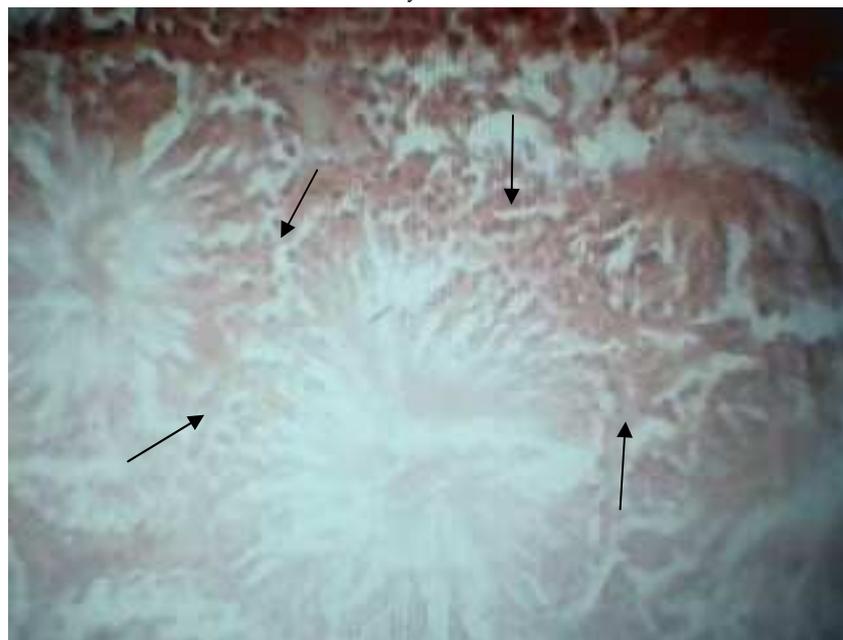
(Haematoxylin and eosin stains; X 400)

**Figure 3:** This figure shows the testis of cryptorchid rat with degenerated epithelium, lumen and interstitium.



*(Haematoxylin and eosin stains; X 400)*

**Figure 4:** This figure shows the testis of cryptorchid rat treated with BLE. The seminiferous epithelium, interstitium were mildly affected



*(Haematoxylin and eosin stains; X 400).*

### Testes Histology

Figure 1 shows the representative sections of the seminiferous tubules of control animals were oval in outline with normal epithelium and intact interstitium. The histological profiles of the testes of animal that were treated with BLE were largely similar to those of the control counterparts (Figure 2). In animals with cryptorchidism there were evidences of degenerative changes in their seminiferous epithelium characterized by

interstitial oedema and vacuolization of the interstitium (Figure 3).The cryptorchid rats treated with BLE showed attenuated seminiferous epithelium and interstitium with mild degenerative changes (Figure 4).

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#### CONFLICT OF INTEREST

No conflict of interest was declared by authors.

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