

CONSUMPTION OF BAY LEAF (A FOOD SPICE) MAY BE A SAFE AND EFFECTIVE TREATMENT FOR MALE INFERTILITY RESULTING FROM PARTIAL LIGATION OF THE LEFT RENAL VEIN IN WISTAR RAT: STUDY SUGGEST

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ABSTRACT

Approximately 10% of all men have varicoceles. Oxidative stress has been implicated in its pathogenesis. Varicocelectomy is not an effective treatment in such cases as it failed to restore fertility after surgery in many patients. Bay leaves are spices used in cooking due to their flavoring capacity and aroma. This study aimed to elucidate the histological and spermographic changes after left experimental varicocele and to evaluate the possible protective role of bay leaf extract. A total of 20 adult male wistar rats were randomly divided into 4 groups (A, B, C and D) with 5 rats in each group. The rats in group A served as the control, Group B and C had a partial ligation of their left renal vein. Group D rats underwent a similar procedure, but varicocele was not induced (sham). Rats in group B in addition had orally treatment with 60 mg/kg of Bay leaf extract daily for 10 weeks after varicocele induction. The animals were sacrificed 10 weeks post operation and histological, sperm parameters and oxidative enzymes were evaluated.

Result showed that the testes of varicocelized models treated with bay leaf extract an improved sperm characteristics and morphology when compared to the untreated

varicocele rats. Supplementation with bay leaf extract in cases of infertility with varicocele may have a protective effect.

KEYWORDS: Varicocele, Infertility, Bay leaf, Oxidative Stress, Sperm count, Histomorphometry

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INTRODUCTION

Varicocele is characterized by abnormal tortuosity and dilation of the veins of the pampiniform plexus within the spermatic cord. It has been the source of much controversy in the medical literature. Although it is notably greater in (25 to 40%) in infertile couples with male factor infertility, the prevalence of varicocele in the general population is about 15-20% (French et al., 2008). Varicoceles most commonly occur on the left side of the scrotum due to absent or incompetent valves in the left internal spermatic vein, and reflux of blood down the vein. Anatomical dissection on men with varicocele demonstrated absence of the valve at the junction of the left renal vein and the internal spermatic vein (Zorgniotti and Sealton, 1998). Engorgement of the left pampiniform plexus and venous collaterals in the scrotum lead to elevated scrotal temperature, which may result in decreased sperm count, abnormal sperm motility and morphology (Koksal et al., 2000, 2002; Romeo et al., 2003). The commonest semen abnormality in patients with varicocele and infertility is poor sperm motility (less than 60% motile forms), followed by abnormal morphology, and to a lesser extent, depression of sperm count below the normal World Health Organization value of 20 million/ml².

Although, decrease in androgen secretion, increased scrotal temperature, reflux of adrenal toxic metabolites, testicular hypoxia have all been implicated in its pathogenesis, the mechanism of action by which varicocele causes degeneration of the testis and sperm

dysfunction is poorly understood (Santoro *et al.*, 2001; Marmar, 2001, Koksai *et al.*, 2007, Shiraishi and Naito, 2005, Shiraishi *et al.*, 2009).

Varicocele models have been created in at least four species of laboratory animals: rat (Turner and Lopez, 1990), dog (Saypol *et al.*, 1981), rabbit (Snydle and Cameron, 1983; Sofikitis and Miyagwa, 1994) and monkeys (Kay *et al.*, 1979; Fussell *et al.*, 1981; Harrison *et al.*, 1986). The varicocele effect was created by partial ligation of the left renal vein/or left testicular vein, leading to sustained partial obstruction with congestion and dilation of the pampiniform plexus and testis vasculature.

A growing number of studies indicated that oxidative stress, which is mainly caused by reactive oxygen species (ROS), plays an important role in sperm dysfunction of patients with varicocele (Smith *et al.*, 1996, Alkan *et al.*, 1997, Barbieri *et al.*, 1999, Hendin *et al.*, 1999, Shiraishi and Naito, 2005). In non oligospermic men with varicocele, spermatozoa, ROS was significantly elevated and the concentration of seminal plasma antioxidant was lower in men with varicocele irrespective of fertility status, and it was concluded that seminal oxidative stress was strongly associated with varicocele and sperm dysfunction (Lenk *et al.*, 1994). Oxygen and oxygen-derived oxidant, responsible for the testicular dysfunction, commonly known as reactive oxygen species (ROS), such as hydrogen peroxide, the superoxide anion, the hydroxyl radical which is generated in biological systems from superoxide anion and hydrogen peroxide by the Haber–Weiss reaction or from hydrogen peroxide by the Fenton reaction and is the strongest of the oxidant species and reacts indiscriminately with nucleic acids, lipids and proteins (LaNasa and Lewis, 1987, Halliwell and Gutteridge, 1990, McClure *et al.*, 1991, Mazzilli *et al.*, 1994.).

Surgery and embolization has been a major techniques used in varicocele repair. Controversy regarding the use has arisen with the first question being: does infertility improve after varicocele repair? Some studies have suggested there is no improvement

(Vermeulen and Vadeweghe, 1984). Many other studies have shown a significant improvement in semen analysis and pregnancy rates after varicocele repair. Another controversy, which seems to be resolving as more series are reported, is whether varicocele embolization is as safe and effective as varicocele surgery.

In recent times, antioxidants from plant materials have been helpful in the fight against free radicals generation [Saalu et al., 2011, Akunna et al., 2012]. Plants like bay leaf (*Laurus nobilis*) which is commonly used as a spice in food have been shown to possess antioxidants antibacterial, antifungal, hypoglycaemic in alloxan-induced diabetes (Kar et al., 2003), anticarcinogenic, antiinflammatory effects (Vandana and Sharma, 2010), and antiulcerogenic properties (Baytop, 1984). Bay leaf extract (BLE) have been used orally to treat gastrointestinal problems, epilepsy, neuralgia and flatulence (Fang et al., 2005). The active components of bay leaves extract are 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 (Gulcin et al., 2002). A significant increase in the levels of lipids and lipid peroxidation products and a decline in antioxidant potential were observed in diabetic rat brain synaptosomes (Pourmorad et al., 2006). The protective capability of bay leaf has been attributed to presence of phytochemicals (Gulcin et al., 2002, Lakshmi et al., 2007, Saalu et al., 2011). The purpose of this present study is to evaluate the protective efficacy of bay leaf extract (BLE) on testicular injury as a result of varicocele.

MATERIALS AND METHOD

Acquisition and Aqueous Extraction of the Bay leaf

Samples of leaves were bought from a local market in Lagos, Nigeria in the month of October, 2012. They were authenticated by a staff in the herbarium of the Department of

Botany, University of Lagos, Nigeria. Bay leaves were dried and the aqueous extraction process was done as described by Elmastaş et al. (2006). The extract was stored in bottles at -20°C until use.

Animal grouping and Varicocele Induction

A total of 20 adult male wistar rats (200–250 g) were used for this study. They were randomly divided into 4 groups (A, B, C and D) with 5 rats in each group. The rats in group A served as the control, Group B and C had a partial ligation of their left renal vein. Group D rats underwent a similar procedure, but varicocele was not induced (sham). Rats in group B in addition had orally treatment with 60 mg/kg of Bay leaf extract daily for 10 weeks after varicocele induction.

Surgical Procedure

Left varicocele was experimentally induced in the rats of groups B and C as described by Turner (2001). In brief, rats were weighed and administered general anesthesia with an intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine (1 mg/kg body weight) (Cam et al., 2004). Then, the left renal vein was dissected with a midline incision. Two knots were tied around the renal vein at a point medial to the insertion of the spermatic vein by using a 20-gauge needle and a 4-0 silk suture. The needle was carefully removed, and the diameter of the left renal vein was reduced by approximately 50% (8, 18) (Figure 1). The rats in group D (sham) underwent similar procedure except that no knots were tied around the needle and renal vein. Group A rats were used as the controls. The animals were sacrificed 10 weeks after operation

Assessment of epididymal sperm parameters

Sperm collection was done by laparotomy, the left and right caudal parts of the epididymis were carefully separated from the testes, minced in 5 ml of Hanks' medium, and incubated for 15 min (Cheng et al., 2006). The diluted sperm suspension (10 ml) was

transferred to the hemocytometer, and the settled sperm were counted with a light microscope at 400× magnification (million/ml) (Seed et al., 1996). The motility assay was conducted by observing the sperm suspension on a slide glass at 37°C. The percentage of motile spermatozoa was determined by counting more than 200 spermatozoas randomly in 10 selected fields under a light microscope (Olympus BX51, Germany), and the mean number of motile sperm $\times 100$ /total number of sperms (Seed et al., 1996, Cheng et al., 2006) was calculated. The sperm morphology was determined by eosin/nigrosin staining. Ten microliters of eosin Y (1%) and nigrosin was added to 50 μ l of sperm suspension. The prepared smear was used after incubation for 45–60 min at room temperature. In each field, 200 sperm were counted under a light microscope (1000×). The sperms were classified on the basis of the following abnormalities: double head, flattened, bent neck, bent tail, and multiple abnormalities (Narayana et al., 2003).

Morphological assessment

This was done as essentially as described by Saalu *et al.*, (2011). 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 μ m 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.

Assessment of biochemical parameters

Catalase activity (CAT) was estimated using the method of Aebi, 1983 as described by Saalu *et al.*, 2006. Superoxide dismutase activity (SOD) was measured according to the method of Winterbourn *et al.*, 1975. Lipid peroxidation (LPO) in the testicular tissue was estimated colorimetrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust, 1978. A principle component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. Glutathione peroxidase activity (GPx) was measured by the method described by Rotruck *et al.*, 1973.

Statistical analysis

The Statistical Package for the Social Sciences (spss) 15.0 software (SPSS Inc., Chicago, IL, USA) was used to analyse data of all patients. Data were expressed in mean \pm SD. Differences between variables with normal distribution were analysed using anova test and between groups were assessed using nonparametric Kruskal–Wallis test. A P-value of ≤ 0.05 was considered statistically significant.

RESULTS

Evaluation of Varicocele and Testicular weight and Volume

As shown in figure 1, all rats with varicocele exhibited a conspicuous dilatation of the left spermatic vein with blood engorgement. In rats of groups A and D, no dilatation of the spermatic vein was observed.

There was a significant ($p < 0.01$) decrease in the testis weight, testis weight/body weight ratio and testis volume of rats in group C when compared to the control group and sham operated group. The group that had bay leaf extract had a statistically significant ($p < 0.01$) increase in the testis geometry (Figure 2).

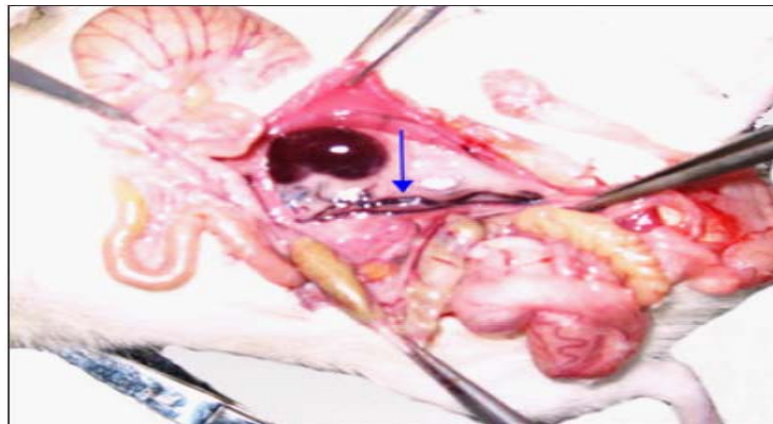


Figure 1: Spermatic vein. Distended spermatic vein in a varicocele rat (arrow).

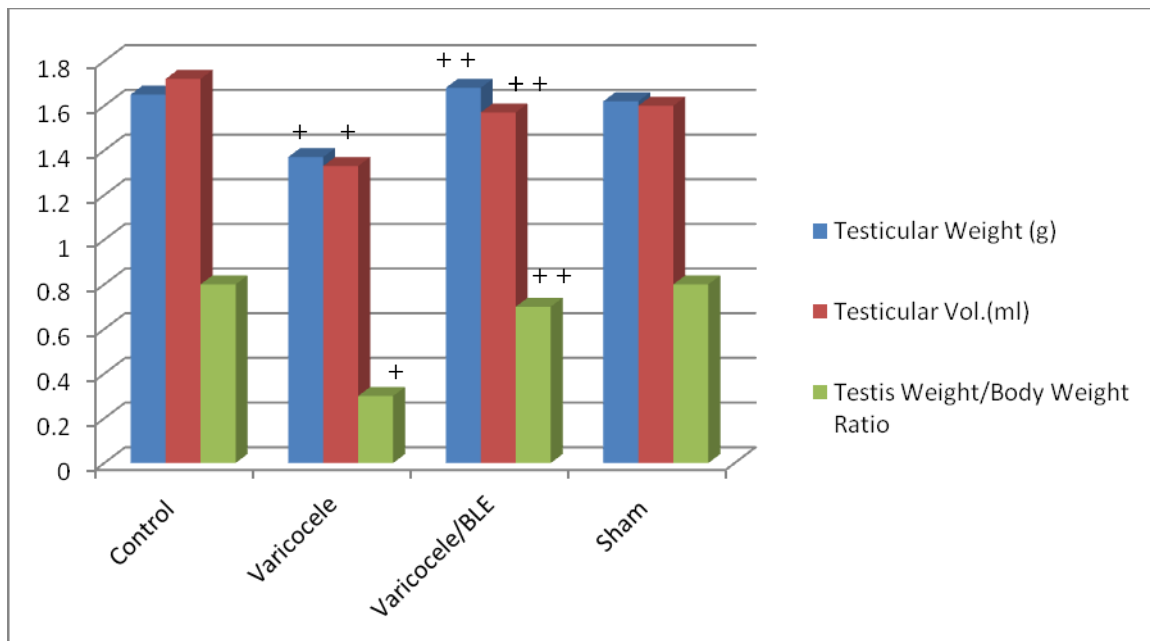


Figure 2: Showing the Testicular Weight, Volume and Testis Weight/body weight differences.

+*P* ≤ 0.05 compared with control and sham group.
 ++*P* ≤ 0.01 compared with the varicocele-alone group.

Epididymal sperm parameters

The untreated varicocele group had a decrease ($P \leq 0.05$) in sperm count, normal morphology and motility in comparison with those of groups A or D (sham and control) (Table1). In group B (treated), the sperm parameters of the left testis improved significantly in comparison with those of the varicocele group ($P \leq 0.01$).

Table1. Epididymal sperm count, graded motility, and morphology in the experimental groups

Variables	Control	Varicocele	Varicocele/BLE	Sham
Count ($\times 10^6 \text{ ml}^{-1}$)	150 (2.5)	123 (3.3)+	140 (2.6)++	145 (4.1)
Normal morphology (%)	87 (7.2)	72 (4.22)+	83 (2.11)	85 (8.4)
Grade 'a' motility (%)	27 (1.4)	10 (8.6)+	23 (4.6)++	26 (9.2)
Grade 'b' motility (%)	35 (8.0)	38 (2.2)	37 (5.0)	36 (7.4)
Grade 'c' motility (%)	15 (2.0)	28 (1.1)	28(7.4)	18 (5.0)

Note. Results are presented as mean (SD).
 $+P \leq 0.05$ compared with control and sham group.
 $++P \leq 0.01$ compared with the varicocele-alone group.

Grade 'a' or rapid motility, Grade 'b' or slow motility, Grade 'c' or non-progressive motility

Testis Morphology

As shown in figure 3, the cross-sections of the seminiferous tubules of control groups of rats were fairly circular in outline with normal seminiferous epithelium and numerous spermatozoa within their lumen and few interstitial blood vessels. The untreated varicocele groups of rat showed destructive changes in their seminiferous tubules and interstitial tissues. There was marked testicular atrophy and interstitial vacuolation (Figure 4). There was significantly improvement in testicular histological profiles of the varicocelized group of

animals that were given 60 mg/kg body weight of Bay leaf extract. These groups of rats showed testicular features that approximated those of the control animals (Figure 5).

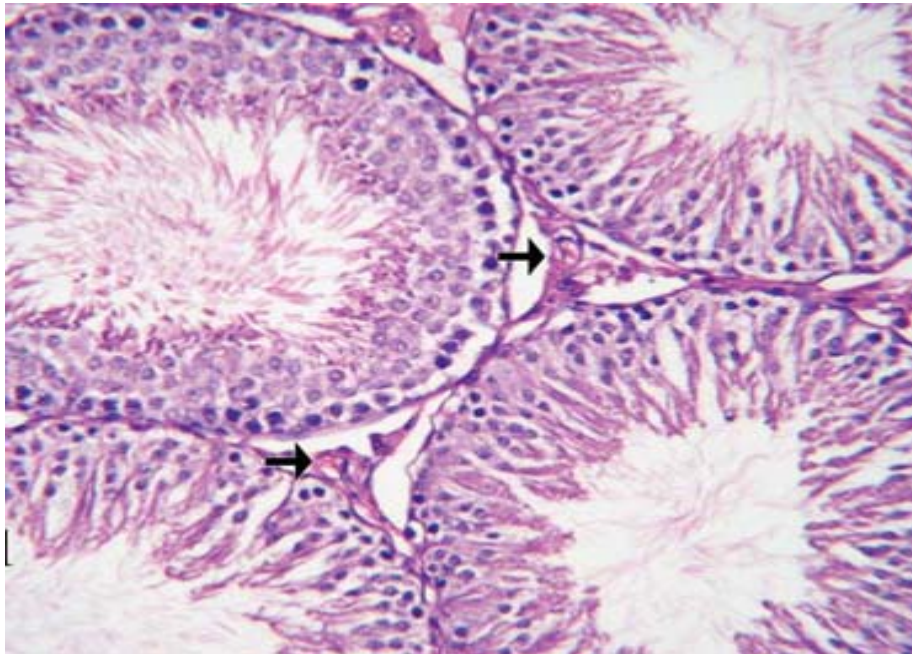


Figure 3: Cross section of the testis of control group (A) of rats showing the structure of the seminiferous tubules with the interstitial tissue. Note the few interstitial blood vessels (arrows). Left H & E (X200)

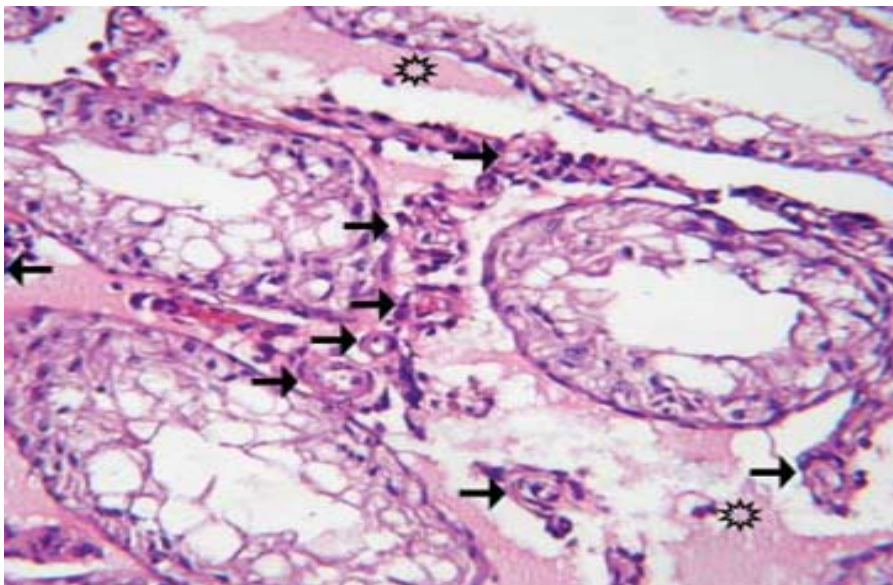


Figure 4: Cross section of the testis of Varicocele group (B) of rats showing the structure of the seminiferous tubules with the interstitial tissue. Note the vacuolated germ cells. Left H & E (X200)

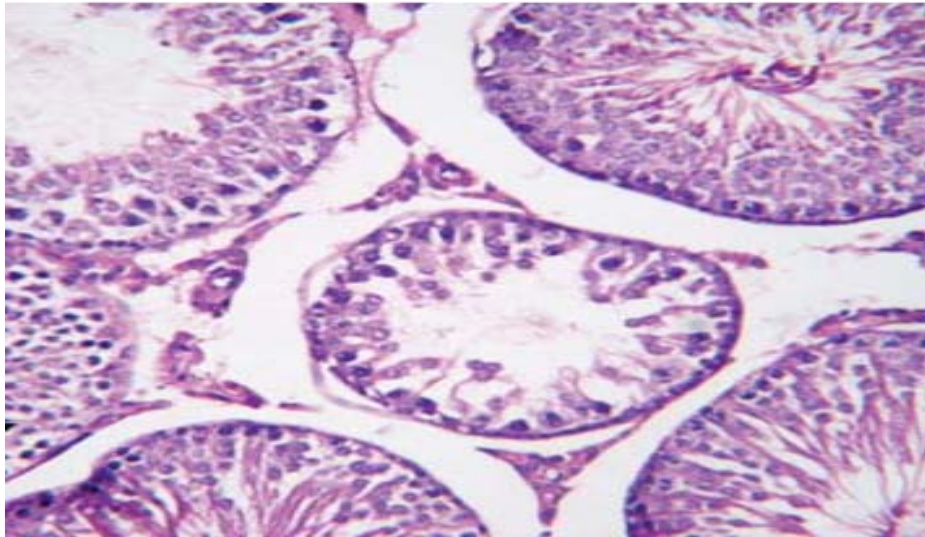


Figure 5: Cross section of the testis of varicocele rat treated with 60mg/kg body weight of Bay leaf extract. Note the variable seminiferous tubules with the interstitial tissue. Left H & E (X200)

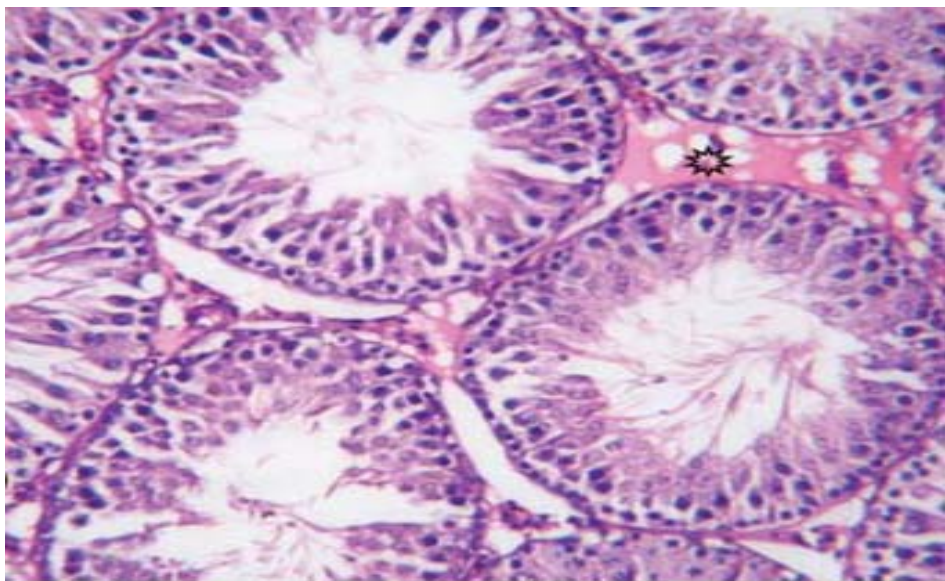


Figure 6: Cross section of the testis of sham operated rat showing the seminiferous tubules with structure similar to the control group. Left H & E (X200)

TESTICULAR OXIDATIVE STRESS

Activities of testicular enzymes SOD, CAT and GPx and Testicular Content of Malondialdehyde (MDA)

As shown in Table 2, untreated varicocele model (Group B) had a significant decrease in SOD activity when compared to control and sham group. Varicocele rats treated with bay leaf extract showed a significantly increased testicular SOD activity which is

comparable to that of the control and sham group. The testicular activities of CAT post treatment with the extract were similar to that of the control values. The untreated varicocele rats, however, had a significant reduction in testicular CAT activity compared to sham and control group of rats. As shown in table 2, the GPx activity of treated varicocele rats approximated that of the control and sham groups of animals. Group B rats, however, had a markedly decreased GPx activity compared to that of sham and control values. Group B rats had a significantly elevated testicular malondialdehyde (MDA) as compared to the control value. However, the varicocele group of rat (group C) that had extract from bay leaf showed a remarkable reduction in their testicular MDA level compared.

Table 1: Showing results for activities of testicular enzymes SOD, CAT and GPx and testicular content of Malondialdehyde (MDA)

Treatment Groups	SOD (u/mg protein)	CAT (u/mg protein)	MDA (nmol/mg protein)	GPx (nmol/mg protein)
Control	6.11 (3.1)	382.21(3.7)	26.7 (5.3)	0.95 (2.1)
Varicocele-Alone	3.31 (4.1)	361.2 (4.5)+	59.3 (4.2)+	0.36(6.1)+
Varicocele/BLE (60mg/kg)	5.13 (5.1)	380.1(5.5)++	29.2 (8.1)++	0.77 (0.1)++
Sham	5.41 (7.1)	375.8 (9.1)	30.3 (7.1)	0.89 (0.4)

+ $P \leq 0.05$ compared with control and sham group.

++ $P \leq 0.01$ compared with the varicocele-alone group. n = 5 in each group.

DISCUSSION

Clinical or sub-clinical varicocele has been shown to cause male infertility (Mehraban et al., 2005). Varicocele, which is the leading cause of male infertility, has been associated with decrease in testicular weight and volume. Our result regarding reduction in testis

geometry is consistent with previous report of considerable decrease in testicular weight due to various derangements (Sofikitis and Miyagawa, 1992, Kisa et al., 2004, *Semercioz et al.*, 2003). Varicocele rat that were treated with 60 mg/kg body weight of Bay leaf extract had a largely preserved testicular weights and volumes, indicating its protective role.

When aqueous extract of Bay leaf (*Laurus nobilis*) was administered to group C rats, the sperm concentration, motility and normal morphology of the sperm improved significantly compared to untreated varicocele group B which had dwindled sperm parameters. Our finding suggests that intraperitoneal administration of extract from Bay leaf (*Laurus nobilis*) in varicocele rat successfully increases the sperm qualities which are in accordance with the report of Bahmanzadeh et al (2008) in which there was a significant improvement observed in all the sperm parameters except for motility varicocele rats (Szabó et al., 1997).

Under certain pathological conditions, such as varicocele, high NO production can cause formation of a highly toxic anion of peroxidation (peroxynitrite ONOO^-) leading to degeneration of the testis. Varicocele has been associated with increased production of ROS (Shiomi et al., 1998, Pagliaro, 2003, Mehraban et al., 2005). Our results are in agreement with the previous reports on other antioxidant agents. Administering vitamin C (well known antioxidants) significantly lower the level of superoxide in varicocele rats (Vakili et al., 2006).

The result in this study showed that untreated varicocele rats had degenerated seminiferous tubules and interstitial oedema. The histological profiles of the varicocele rats that were treated with Bay leaf (*Laurus nobilis*) extract were almost similar to those of the control and sham models which had completely differentiated seminiferous tubules with numerous spermatozoa and normal and intact epithelium. Our findings are in accordance with

our previous report implicating varicocele in testicular degeneration (Kisa et al., 2004, Menna and Abdel-Dayem, 2004, Jiang et al., 2008).

Histological profile of the treated varicoceles models were similar to that of the control, however there have been no studies that clearly demonstrate that testicular histology can predict success after varicocele treatment (Cocuzza et al., 2008).

In 1994, *Faizi et al.* reported that enhancing the antioxidant system levels can favour reproductive potentials. The findings in our study confirmed the role of reactive oxygen species in the pathogenesis of varicocele being in accordance with previous reports (Alvarez et al., 1987, Weese et al., 1993, Cocuzza et al., 2008) Positive changes evidenced in our study might have been as a result of the ability of flavonoids in bay leaf (*Laurus nobilis*) to scavenge hydroxyl radicals hence inhibiting lipid peroxidation (Bahmanzadeh et al., 2008). Although Bay leaf (*Laurus nobilis*) has been shown to have antioxidant properties (Gulcin et al., 2002, Lakshmi et al., 2007), these properties have not been reported in varicoceles animal model.

Therefore aqueous extract of bay leaf (*Laurus nobilis*) could be useful for the treatment of varicocele and possibly other clinical conditions involving excess free radical production.

REFERENCE

Aebi H. (1983) Catalase. In methods of enzymatic Analysis, Bergmeyer, H.Ed. Verlag Chemical, Weinheim 3: 273.

Akunna GG, Saalu LC, Ogunmodede OS, Ogunlade B, Bello AJ. (2012) Aqueous Extract of Date Fruit (*Phoenix Dactylifera*) Protects Testis against Atrazineinduced Toxicity in Rat. World Journal of Life Sciences and Medical Research 2(2):100-8.

Alvarez JG, Touchstone JC, Blasco L, Storey BT. (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. *J Androl.*, 8:338-48.

Bahmanzadeh M, Abolhassani F, Amidi F, Ejtemaiemehr Sh, Salehi M, Abbasi M. (2008) The effects of nitric oxide synthase inhibitor (L-NAME) on epididymal sperm count, motility, and morphology varicocele rat. *DARU.* 16(1): 23–28.

Baytop T. (1984) *Therapy with Medicinal Plants in Turkey.* No. 3255, Publications of Istanbul University, Istanbul, 1984; p. 194.

Buege, J.A., and Aust, S.D. (1978). Microsomal Lipid Peroxidation. In: *Methods in Enzymology.* Fleischer, S., Packer L., (Editors), New York: *Academic Press.* 302-330.

Cam K, Simsek F, Yuksel M, Turkeri L, Haklar G, Yalcin S, Akdas A. (2004) The role of reactive oxygen species and apoptosis in the pathogenesis of varicocele in a rat model and efficiency of vitamin E treatment. *Int J Androl.*, 27(4): 228–233.

Cheng D, Zheng XM, Li SW, Yang ZW, Hu LQ. (2006) Effects of epidermal growth factor on sperm content and motility of rats with surgically induced varicoceles. *Asian J Androl.*, 8(6): 713–717.

Cocuzza M, Athayde KS, Agarwal A, Pagani R, Sikka SC, Lucon AM, et al. (2008) Impact of clinical varicocele and testis size on seminal reactive oxygen species levels in a fertile population: a prospective controlled study. *Fertil Steril.*, 90:1103-1108.

Elmastas M Gulcin İ, Beydemir Ş, Kufrevioğlu Oİ, Aboul-Enein HY. *Anal. Lett.*, 39- 47.

Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH. (1994) Isolation and structure elucidation of new nitrile, mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *J. Nat. Prod.*, 57: 1256-1261.

Fang F, Shengmin S, Kuang YC, Alexander Gossau, Chi-Tang Ho, Robert T, Rosen. (2005) Isolation and identification of cytotoxic compounds from Bay leaf (*Laurus nobilis*) *Food Chemistry.* 93 497–501.

French DB, Desai NR, Agarwal A (2008). Varicocele repair: Does it still have a role in infertility treatment? *Curr. Opin. Obstet. Gynecol.* 20(3): 269-274.

Fussell, E.N., Lewis, R.W., Roberts, J.A. et al. (1981) early ultra structural findings in experimentally produced varicocele in the monkey testis, *J. Androl.*, 2, 111-119

Halliwell B, Gutteridge (1998) JMC, editors. *Free Radicals in Biology and Medicine*, 3rd ed. Oxford: Oxford Science.

Harrison, R.M., Lewis, R.W and Roberts, J.A. (1986) Pathophysiology of varicocele in nonhuman primates: long term seminal and testicular changes. *Fertil. Steril.*, 46, 500-509

Jiang YG, Peng T, Luo Y, Li MC and Lin YH. (2008) Resveratrol reestablishes spermatogenesis after testicular injury in rats caused by 2, 5-hexanedione. *Chin. Med. J. (Engl)* Jul 5;121(13):1204-1209.

Kar A, Choudhary B K and Bandyopadhyay N G, (2003) comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats, *J ethno pharmacology.* 2003;84-105

Kay, R, Alexander, NJ. And Baugham, W.L (1979) Induced varicocele in Rhesus monkeys. *Fertil. Steril.*, 31, 195-199.

Kisa U, Basar MM, Ferhat M, Yilmaz E, Basar H, Caglayan O, et al. (2004) Testicular tissue nitric oxide and 23. thiobarbituric acid reactive substance levels: evaluation with respect to the pathogenesis of varicocele. *Urol Res.* 32(3): 196–199.

Koksal IT, Ishak Y, Usta M, Danisman A, Guntekin E, Bassorgun IC, et al. (2007) Varicocele-induced testicular dysfunction may be associated with disruption of blood-testis barrier. *Arch Androl.* 53:43-48.

Kourounakis AP, Galanakis D, Tsiakitzis K. (1999)*Drug Develop. Res.*, 1:47-9.

Lakshmi SD, Kannappan S, Anuradha. (2007) Evaluation of in vitro antioxidant activity of Indian bay leaf, *Cinnamomum tamala* (Bunch.-Ham.) T. Nees and Eberm using rat brain synaptosomes as model system. *Indian J of exp Bio.*, vol. 45, pp. 778-784.

LaNasa JA Jr, Lewis RW. Varicocele and its surgical management. (1987) *Urol Clin North Am.*, 14:127-135.

Lenk S, Fahlenkamp D, Gliech V, Lindeke A. (1994) Comparison of different methods of treating varicocele. *J Androl* 15:34S-37S.

Marmar JL. (2000) The pathophysiology of varicoceles in the light of current molecular and genetic information. *Hum Reprod Update*, 7:461-72.

Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F. (1994) Superoxide anion in human semen related to seminal parameters and clinical aspects. *Fertil Steril.* 62:862-68.

Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F. (1994) Superoxide anion in human semen related to seminal parameters and clinical aspects. *Fertil Steril.*, 62:862-68.

McClure RD, Khoo, D, Jarvi K, Hricak H. (1991) Subclinical varicocele: The effectiveness of varicolectomy. *Journal of Urology*, 145:789-791.

Mehraban D, Ansari M, Keyhan H, Sedighi Gilani M, Naderi G, Esfehani F. (2005) Comparison of nitric oxide concentration in seminal fluid between infertile patients with and without varicocele and normal fertile men. *Urol J.*, 2(2): 106–110.

Mehraban D, Ansari M, Keyhan H, Sedighi Gilani M, Naderi G, Esfehani F. (2005) Comparison of nitric oxide concentration in seminal fluid between infertile patients with and without varicocele and normal fertile men. *Urol J.*, 2(2): 106–110.

Menna M. Abdel-Dayem. (2009) Histological and Immunohistochemical Changes in the Adult Rat Testes after Left Experimental Varicocele and Possible Protective Effects of Resveratrol, *Egypt. J. Histol. Vol. 32, No. 1, June, 81 – 90*

Pagliari P. (2003) Differential biological effects of products of nitric oxide synthase: it is not enough to say NO, 24. in *Life sciences. Else.*, 73(17): 2137–2149.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*. 179: 588-590

Saalu LC, Osinubi AA, Akinbami AA, Yama OE, Oyewopo AO, Enaibe BU. (2011) Moringa oleifera Lamarck (drumstick) Leaf Extract Modulates the Evidences of Hydroxyurea - Induced Testicular Derangement. *Int. J. Applied Res. in Natural Prod.*,4(2):32-45.

Saalu, L. C., Togun, V. A., Oyewopo, A. O. and Raji, Y. (2006).Artificial cryptorchidism and the moderating effect of melatonin in Sprague-Dawley rats. *J.Appl. Sel.* 6(12), 1: 2889-94.

Santoro, G.; Romeo, C.; Impellizzeri, P.; Cutroneo, G. & Trimarchi, F. (2000) Nitric oxide synthase patterns in normal and varicocele testis in adolescents. *BJU Int.* 88:967-73.

Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL , Perreault SD 20. , Schrader S, Seyler D, Sprando R, Treinen KA, Veeramachaneni DN, Wise LD. (1996)Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. ILSI Risk Science Institute Expert Working Group on Sperm Evaluation. *Reprod Toxicol.*, 10(3): 237–244.

Semercioz, A.; Onur, R.; Ogras, S. & Orhan, I. (2003) Effect of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuro Endocrinol. Lett.*, 24(1/2):86-90.

Shiomi M, Wakabayashi Y, Sano T, Shinoda Y, Nimura Y, Ishimura Y, Suematsu M. (1998) Nitric oxide suppression reversibly attenuates mitochondrial dysfunction and cholestasis in endotoxemic rat liver. *Hepatology*, 27(1): 108–115.

Shiraishi K, Takihara H, Matsuyama H. (2009) Elevated scrotal temperature, but not varicocele grade, reflects testicular oxidative stress-mediated apoptosis. *World J. Urology*.

Shiraishi K, Takihara H, Matsuyama H. (2009) Elevated scrotal temperature, but not varicocele grade, reflects testicular oxidative stress-mediated apoptosis. *World J Urol*, 2009.

Snydle, F.E. and Cameron, D.F. (1983) Surgical induction of varicocele in the rabbit. *J. Urol.*, 130, 1005- 1009.

Sofikitis, N. & Miyagawa, I. (1992) Experimental models for the study of varicocele: A selected review. *Jpn. J. Fertil. Steril.*38:168-77.

Sofikitis, N. & Miyagawa, I. (1992) Experimental models for the study of varicocele: A selected review. *Jpn. J. Fertil. Steril.*1992; 38:168-77.

Szabó C, Ferrer-Sueta G, Zingarelli B, Southan GJ, Salzman AL, Radi R. (1997) Mercaptoethylguanidine and 30. guanidine inhibitors of nitric-oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage. *J Biol Chem.*, 272(14): 9030–9036.

Turner TT. The study of varicocele through the use of animal models. *Hum Reprod Update*. 2001; 7(1): 18. 78–84.

Turner, T.T and Lopez, T.J. (1990) Testicular blood flow in peri-pubertal and old rats with unilateral experimental varicocele and investigation into the mechanism of the bilateral response to unilateral lesion. *J. Urol.*, 144, 1018-1021

Vakili A, Nekooeian A, Dehghani GA. (2006) Aminoguanidine reduces infarct volume and improves neurological dysfunction in transient model of focal cerebral ischemia in rat. *DARU.*, 14(1): 126–130.

Vandana Gupta, Mukta Sharma. (2010) Protective Effect of Cinnamomum tejapata on Lipid Peroxide Formation in Isolated Rat Liver Homogenate. *Curr Research Jnal of Bio Sci.* 2(4):246-9.

Vermeulen A, Vadeuweghe M. (1984) Improved fertility after varicocele correction: fact or fiction? *Fertility and Sterility*, 42:249-256.

Weese DL, Peaster ML, Himsl KK, Leach GE, Lad PM, Zimmern PE. (1993) Stimulated reactive oxygen species generation in the spermatozoa of infertile men. *J Urol.*, 149:64-7

Winterbourn, C., Hawkin, R., Brian, M. and Carreli, R. (1975) The estimation of red cell superoxide dismutase activity. *J. Lab. Clin.Med.* 85, 337-340.

Zorgniotti AW, Sealfon AI: Studies in temperature, human semen quality and varicocele. *J. Reprod Steril* 1988; 82:563.