First International Journal of Andrology
ANDROLOGIA

INVITED REVIEW

Phytoandrogenic properties of *Eurycoma longifolia* as natural alternative to testosterone replacement therapy

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Keywords
Ageing males' symptoms—*Eurycoma longifolia*—herbal medicine—hypogonadism—testosterone replacement therapy

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Accepted: November 11, 2013

doi: 10.1111/and.12214

Summary

The testosterone deficiency syndrome (TDS) is characterised by numerous symptoms, including low libido, increased fat mass, fatigue, erectile dysfunction or osteoporosis, and up to 80% of men will experience some kind of ageing males' symptoms. This is caused by the age-depending decline in serum testosterone levels with concentrations being about 40–50% lower in men older than 60 years compared with young men. This significant decline in testosterone levels is further closely linked with medical conditions such as obesity, metabolic syndrome, diabetes or hypertension. The conventional way of treating TDS is the testosterone replacement therapy (TRT), for which preparations are on the market. Apart from the beneficial effects of TRT, significant adverse side effects have been described, and prostate cancer (PCa) as absolute contraindication is debated. *Eurycoma longifolia* (Tongkat Ali; TA) is natural alternative to TRT and has been shown to restore serum testosterone levels, thus significantly improving sexual health. This includes significant positive effects on bone health and physical condition of patients. In addition, a significant antihyperglycaemic effect and cytotoxicity against PCas cells has been shown. Thus far, at therapeutic concentrations, no significant side effects of the treatment were obvious. Therefore, TA might be a safe alternative to TRT.

Introduction

Hypogonadism in men is defined as the inadequate functioning of the testicles and can be distinguished in primary (hypergonadotropic) and secondary (hypogonadotropic) hypogonadism. While the first refers to testicular causes of the hypogonadism, the latter refers to hypothalamic or pituitary disorders underlying the testicular malfunction with androgen deficiency. The clinical picture of the testosterone deficiency eventually depends on the time when it appears, during the foetal period, puberty or in adulthood, as well as its extent (Jockenhövel, 2004). Hypogonadism is caused by ageing and diseases such as Klinefelter's syndrome, orchitis and pituitary and hypothalamic dysfunction (Dandona & Rosenberg, 2010) and is eventually resulting in a reduced testosterone levels.

In the context of this review, we focus on hypogonadism in older men, where it is also referred to as 'andropause' (Matsumoto, 2002), 'androgen deficiency in the ageing male' (ADAM) (Morales & Tenover, 2002), 'partial androgen deficiency in the ageing male' (PADAM) (Frajese *et al.*, 2005), 'testosterone deficiency syndrome' (TDS) (Morales *et al.*, 2006) or 'late-onset hypogonadism' (LOH) (Gooren, 2009). Other names used for this condition are 'male menopause', 'viropause' or 'male climacteric'. While the terms 'male menopause', 'viropause' and 'andropause' are linguistically inappropriate because men do not have a menses nor there is a loss of virility or any complete cessation of testicular function including testosterone secretion that would justify the term 'pause', 'male climacteric' does not specifically address the ageing male syndrome with regard to the testosterone levels (Matsumoto, 2002). On the other hand, LOH refers to late age decline of androgen, which is also not quite correct as this decline starts at middle age. Therefore, we prefer TDS as this term characterises the situation with its defined clinical and biochemical symptoms best, in middle age and elderly men. This term is also recommended by the International Society of Andrology (ISA), the International Society for the Study of Ageing Male

(ISSAM), the European Association of Urology (EAU), the European Academy of Andrology (EAA), and the American Society of Andrology (ASA) (Wang *et al.*, 2009).

Among other symptoms, TDS is characterised by symptoms of low libido, increased fat mass, decreased muscle mass, loss of concentration, erectile dysfunction (ED), depression, and decreased bone mineral density and by a deficiency in serum testosterone levels (Schulman *et al.*, 2009). Until men reaching 30 to 40 years of age, the levels of bioavailable testosterone remain fairly constant. However, as from about 40 years of age, serum testosterone concentrations in men decline with annual rates between 0.4% and 2.6% for total testosterone and 0.87% and 1.7% for free testosterone (Harman *et al.*, 2001; Feldmann *et al.*, 2002; Henkel *et al.*, 2005; Kaufman & Vermeulen, 2005). Eventually, this decline results in serum testosterone levels being 40–50% lower at the age of 60 than at young age.

In men as from about the age of 50 years, this decline is, apart from significant morphological changes in the testes (Holstein, 1986), due to a marked decrease in the number and function of Leydig cells (Neaves *et al.*, 1985; Johnson, 1986) leading to a reduced basal state testosterone production, which appears not to respond to LH- or hCG- stimulation (Longcope, 1973; Harman & Tsitouras, 1980; Veldhuis *et al.*, 2012). Considering that LH levels in ageing men either do not change or are slightly raised (Wu *et al.*, 2008; Surampudi *et al.*, 2012), the decreased mitochondrial steroidogenesis (Takahashi *et al.*, 1983) is obviously not due to reduced LH levels, but to a reduced production of testosterone caused by significant deficits in LH receptor number, cAMP production, steroidogenic acute regulatory (STAR) protein and translocator protein (TSPO) cholesterol transport as well as reduced activity of steroidogenic enzymes in mitochondria and smooth endoplasmic reticulum (Chen *et al.*, 2009). Contributing to this is the age-related increase in the serum concentration of sexual hormone-binding globulin (SHBG) (Gyllenborg *et al.*, 2001; Feldmann *et al.*, 2002) and aromatase activity (Ishunina *et al.*, 2005).

Consequently, more than 20% of men older than 60 years of age present with low total [<10.5 nM (300 ng dl^{-1}); definition of hypogonadism] and free [<1.7 nM (5 ng dl^{-1})] testosterone (Kaufman & Vermeulen, 2005; Araujo *et al.*, 2007) and even younger men can be plagued (Matsumoto, 2003). Yet, in the light of many men not coming to medical examinations for the reasons of self-perceived deflated ego and the fact that determining testosterone levels is not routinely tested, these numbers might even be too low. Therefore, clinically significant TDS is currently under-diagnosed (Schulman *et al.*, 2009). In addition, TDS can result in reduced quality of life and adversely affect the bodily functions as it is closely linked with other medical conditions such as obesity, metabolic syndrome, diabetes, insulin resistance, glycaemic control, hypertension, rheumatoid arthritis and osteoporosis (Feeley & Traish, 2009; Schulman *et al.*, 2009; Leisegang *et al.*, 2012). In addition, several reports show that compared with normal testosterone levels, patients with low serum testosterone levels had, depending on the study, up to 88% increased mortality risk (Shores *et al.*, 2006; Laughlin *et al.*, 2008).

Testosterone replacement therapy: benefits, disadvantages and contraindications in Western medicine

Testosterone deficiency syndrome is treated with testosterone replacement therapy (TRT). Short-acting testosterone esters and long-acting preparation, testosterone undecanoate (Nebido®, Bayer Schering, Pharma AG, Berlin, Germany), testosterone implants that are inserted into a deep subcutaneous position, testosterone patch such as the Andropatch® (GlaxoSmithKline, Middlesex, UK), testosterone in an alcohol-based gel preparation and testosterone tablets are some of the examples of TRT used (Seal, 2009). Testosterone treatment has several benefits such as improving sexual desire and function (Tenover, 1997), increase bone mineral density (Amory *et al.*, 2004; Aminorroaya *et al.*, 2005), improve mood, energy and quality of life (Lunenfeld & Nieschlag, 2007), change body composition and improve muscle mass and strength (Strollo *et al.*, 2013), improve cognitive function (Janowsky *et al.*, 1994; Wolf *et al.*, 2000) and improving metabolic syndrome and type-2 diabetes and cardiovascular disease (Kapoor *et al.*, 2006; Corona *et al.*, 2011a,b; Strollo *et al.*, 2013). Due to the multifactorial causes of ED, ageing men with ED might require a combination therapy of TRT with phosphodiesterase-5 (PDE-5) inhibitors such as sildenafil or tadalafil as about 30% of the patients with ED treated with PDE-5 inhibitors do not respond (Buvat *et al.*, 2011; Khera *et al.*, 2011). Thus, the benefits of TRT in a variety of clinical conditions has been shown and confirmed in a number of studies.

Apart from these clear benefits of TRT, the form of testosterone application, intramuscular (e.g. Delatestryl®, SAB-Pharma Inc, Boucherville, QC, Canada; Nebido®), subcutaneous (Testopel®, Bartor Pharmacal, Rye, NY, USA), transdermal (e.g. Androderm®, Watson Pharmaceuticals Inc, Corona, CA, USA; Androgel®, AbbVie Inc., North Chicago, IL, USA), buccal (Striant®, Mipharm S.p.A, Milan, Italy) or oral (e.g. Andriol®) are either not approved in various countries (e.g. Andriol®), cause pain when applying (e.g. Delatestryl®), require surgical insertion (Testopel®), are messy, cause contact dermatitis or have transmission risk to other people (e.g. Androgel®,

Androderm®) (Seal, 2009; McGill *et al.*, 2012). While intramuscular application gives good results with smooth testosterone profiles (Nebido®), a problem is the compliance by men. On the other hand, testosterone tablets, which are easy and convenient to take, are rapidly absorbed in the intestine, but only result in low serum testosterone concentrations and large hormone level swings (Seal, 2009; McGill *et al.*, 2012).

Furthermore, TRT has been associated with adverse side effects, and therefore, a number of contraindications are given. Considering that testosterone induces erythropoietin production causing an increase in haemoglobin and erythrocyte concentrations (haematocrit), which in turn increases the risk of a stroke or cardiovascular events due to increased blood viscosity (Krauss *et al.*, 1991; Gagnon *et al.*, 1994; Fernandez-Balsells *et al.*, 2010). Erythrocytosis is the most frequent cause of discontinuation of TRT (Calof *et al.*, 2005). According to the Endocrine Society's recommendations, hematocrit levels should be kept below 50% in patients with TRT (Bhasin *et al.*, 2010).

Hepatic dysfunction has been reported with incidences of up to 4.4% in patients treated with testosterone (Van Kesteren *et al.*, 1997), and complete hepatic failure, liver toxicity and liver tumour have been reported in patients treated with methyl testosterone (Wilder, 1962; Westaby *et al.*, 1977; Gurakar *et al.*, 1994). TRT has been shown to exacerbate obstructive sleep apnoea and increased the occurrence of cardiac arrhythmia that is associated with this condition (Matsumoto *et al.*, 1983; Sandblom *et al.*, 1983; Bhasin, 2003).

With regard to the prostate, exogenous testosterone administration is thought of stimulating growth of prostate cancer (PCa) (Holmäng *et al.*, 1993) and worsens symptoms of benign prostatic hypertrophy (Siiteri & Wilson, 1970) as PCa is androgen-dependent tumour. Therefore, according to the recommendations of the Endocrine Society clinical practice guidelines (Bhasin *et al.*, 2010), PCa is a contraindication for TRT, although this view is controversially discussed as it is based on the observation of Huggins & Hodges (1941) that metastatic PCa regresses after castration. There are also reports indicating that serum testosterone levels are associated with PCa (Gaylis *et al.*, 2005; Pierorazio *et al.*, 2010). On the other hand, these reports were severely criticised for being flawed (Morgentaler *et al.*, 2006, 2010). Yet several studies failed to establish a clear link between an increased risk of PCa development, progression or recurrence in patients treated with testosterone (Isbarn *et al.*, 2009; Traish *et al.*, 2011). This failure to find a distinct link between PCa growth and serum testosterone levels has recently been explained with the concept of a saturation model, according to which serum androgen levels below a not yet clearly defined point of maximum testosterone binding to the androgen receptor (AR) in prostate cells will result in considerable changes in PCa growth. Once a maximum binding is achieved, further increased androgen concentrations will have no or only little effect (Morgentaler & Traish, 2009). Nevertheless, it is recommended that patients showing elevated levels of prostate-specific antigen (PSA), increased PSA velocity or suspicious rectal examination results undergo a prostate biopsy (McGill *et al.*, 2012).

Eurycoma longifolia a natural alternative to testosterone replacement therapy

Eurycoma longifolia is popularly known as Tongkat Ali (TA) in Malaysia, Pasak Bumi in Indonesia and Cay Ba Bihn in Vietnam (Goreja, 2004). It is a medium size slender tree from the family of Simaraubaceae reaching 10 m in height, often unbranched with reddish brown petioles (Zhari *et al.*, 1999). It is largely found as an under-storey growth of lowland forests in Peninsula Malaysia and other Southeast Asian regions (Joseph *et al.*, 2005). The root of the plant is traditionally boiled and consumed as a tonic for aphrodisiac effects and energy in men. *Eurycoma longifolia (El)* is reputed to be a cure for many conditions including malaria, high blood pressure, fatigue, migraine, fever, arthritis, improvement of testosterone production and symptoms of impotence, loss of desire/libido, improved physical and mental performance, enhanced energy levels, endurance and stamina, improved skin and muscle tone and enhancement of the immune system (Goreja, 2004; Ismail *et al.*, 2012; Tambi *et al.*, 2012). Due to the many traditional and scientific benefits, there has been a demand for *El* products with over 202 *El* products registered with the National Pharmaceutical Control Bureau of Malaysia (NPCB, 2013). *Eurycoma longifolia (El)* is now currently sold as Traditional Medicine in Malaysia. TA products are available either in the form of raw crude powder of the root, as capsule as a singular ingredient or mixed with other herbs and as an additive mixed with coffee.

There are currently 65 compounds isolated from TA (Kuo *et al.*, 2003). The plant parts are rich in bioactive compounds eurycomaoside, eurycolactone, eurycomalactone, eurycomanone and pasakbumin-B whereby the alkaloids and quassinoids form a major portion (Bhat & Karim, 2010). The quassinoid compound eurycomanone is used as a marker in standardised water extract according to SIRIM standards (Malaysian Standards, 2011) and has been found to increase testosterone levels and increase the production of sperm in animal models (Ang & Sim, 1998a,b; Zanoli *et al.*, 2009; Low *et al.*, 2013b). The extract has been described as an adaptogen (Tambi & Kadir, 2006) and a traditional antiageing remedy, par-

ticularly for ageing men to improve age-related reduced energy level, mood, sexual function and libido (Adimoelja, 2000; Cyranoski, 2005).

There appears to be more than one mechanism of action in the increase of serum testosterone levels upon supplementation with *El*. A bioactive, patented peptide compound of 4.3 kDa was isolated from water extracts of *El*, which increased testosterone levels and increased sperm count and motility in animal models (Sambandan *et al.*, 2006). The mechanism of action was suggested as by the enhancement of the biosynthesis of various androgens by the peptides found in water extracts of TA roots (Ali & Saad, 1993). The term eurypeptides that was coined to describe the peptide was shown to activate the CYP17 (17 α-hyroxylase/17, 20 lyase) enzyme to enhance the metabolism of pregnenolone and 17-OH-pregnenolone to yield more dehyroepiandrosterone (DHEA). Progesterone and 17-OH-progesterone is further metabolised to 4-androstenedione and testosterone. In a recent report, Low *et al.* (2013a) describe an enhanced testosterone production by Leydig cell explants via the inhibition of phosphodiesterase and aromatase by eurycomanone, a major quassinoid compound present in *E. longifolia* root extract. Some possible mode of action for the increase in testosterone levels was described by Pihie (2004) whereby TA was found to increase cAMP levels, thus enhancing glucose utilisation, which may be attributing to the energy increasing effects.

In another recent study (Low *et al.*, 2013b), using quassinoid-rich fractions of TA, the extract induced testosterone synthesis and elevated LH and FSH but reduced oestrogen levels in the plasma providing evidence that *El* treatment may down-regulate the oestrogen-mediated feedback effect on LH and FSH secretion in the hypothalamic–pituitary–gonadal axis (Prakash, 2007). Hence, a reduction in oestrogen would lead to an elevation of the gonadotropins LH and FSH leading to an increase in testosterone.

While testosterone levels were significantly increased in the plasma of male rats when treated with a dosage of 25 mg kg^{-1} body weight quassinoid-rich fractions, a higher dosage of 50 mg kg^{-1} resulted in significantly lower testosterone production by Leydig cell explants after the *in vivo* treatment (Low *et al.*, 2013b), revealing possible adaptogenic activity of the herb (Tambi, 2006, 2009). In addition, despite the fact that this low concentration (25 mg kg^{-1}) of the extract stimulated higher LH secretion than the high dose (50 mg kg^{-1}), the serum oestrogen levels showed an inverse picture. These data suggest that the quassinoid-rich extract of TA improves male fertility and serum testosterone levels by affecting the hypothalamus or pituitary (Low *et al.*, 2013b). This indirect action of TA can be confirmed by data from Erasmus (2013) who showed an increase in the testosterone production of 4% by TM3-Leydig cells *in vitro* at a concentration of 50 μg mg^{-1} of the extract. This mode of action might also be in line with the view that the *E. longifolia* root extract must rather be seen as testosterone 'maintainer' or 'restorer' by releasing testosterone from sex hormone–binding globulin (SHBG) (Chaing *et al.*, 1994; Talbott *et al.*, 2013). This idea can be supported by recent data by Henkel *et al.* (2013) showing that treatment with 400 mg TA extract per day over 5 weeks resulted in a decreased concentration of SHBG.

Effect on reproductive system

Low fertility rates can be attributed to many causes, some being hormonal imbalances (Pinto *et al.*, 2008) and testosterone treatment has been shown to improve fertility rates in subjects with idiopathic fertility in a double-blind study (Gregoriou *et al.*, 1993). The effect of TA in reducing the inhibitory effects of oestrogen on spermatogenic cells were demonstrated where the spermatogenic cell counts and the percentage of motile sperms in the TA-treated group and the *El* combined with estradiol-treated group increased significantly compared with the estradiol-treated group. This demonstrated the protective and ameliorative actions of TA (Wahab *et al.*, 2010). TA may have a remedial action when excess oestrogen or deficiency in testosterone is the cause for infertility (Tambi & Imran, 2010; Wahab *et al.*, 2010). In fact, in female rats with induced irregular oestrous cycle and cystic follicles, treatment with standardised quassinoid-rich extract of *E. longifolia* (TAF273) ameliorated the reproductive disorder (Abdulghani *et al.*, 2012).

Improvements in reproductive health were also demonstrated in humans. In a study that investigated 75 men with idiopathic infertility, TA supplementation of 200 mg day^{-1} improved semen profiles by increasing higher semen volumes, sperm concentrations, the percentage of normal sperm morphology and sperm motility (Tambi & Imran, 2010). The standardised aqueous extract of TA called Physta® significantly improved the sperm quality in these patients, allowing for 11 (14.7%) spontaneous pregnancies. An improvement in volumes of seminal fluid and sperm motility was also demonstrated in a subpopulation with low baseline values in another study (Ismail *et al.*, 2012).

Effect of TA in sexual health

Tongkat Ali has been traditionally used as an aphrodisiac for centuries. Since this revelation, many scientific researches were carried out to qualify this traditional claim. Therefore, the effect of TA as an aphrodisiac was tested in several studies on sexually experienced, middle-

aged, sexually sluggish and old and sexually naive males rats, each showing an improvement in sexual activities. An increase in libido was observed in a dose-dependent manner, measured by mounting frequencies in sexually experienced male rats (Ang & Sim, 1997). Tongkat Ali extract also increased sexual orientation of sexually experienced male rats towards receptive female rats measured by sexual interest such as anogenital sniffing, licking and mounting, restricted environmental response, self-interest such as genital grooming of themselves and decreased interest in external environment (Ang & Sim, 1998b). Ang and Lee (2002) studied the sexual motivation of middle-age and retired breeder rats and showed significantly increased sexual orientation towards receptive females. On the other hand, mounting hesitation decreased when compared to control even though an electrical copulation cage was used (Ang *et al.*, 2003).

Tongkat Ali supplementation improved the vigour in animal models by increasing sexual vigour in sluggish rats (Ang *et al.*, 2003) and in middle-age rats that were no longer used for breeding. TA supplementation reduced hesitation in mounting females rats (Ang & Lee, 2002), in what can be inferred to as a willingness towards physical functioning with an increase in sexual motivation.

It was also noticed that there was a momentary enhancement in the percentage of male rats responding to the right choice after chronic administration of 0.5 g kg^{-1} in sexually sluggish old male rats. Tongkat Ali administration had aphrodisiac effects demonstrated by the act of yawning and stretching which is an indication of sexual arousal, in a dose dependent manner (Ang *et al.*, 2004). Tongkat Ali administration in sexually inexperienced rats increased experience of treated rats significantly when compared to the control group (Ang & Sim, 1998b). A pro-androgenic effect was demonstrated on laevator ani muscle of castrated and uncastrated male rats (Ang & Cheang, 2001) by an increase in its weight (enlargement) upon TA treatment suggesting for the first time the scientific mechanism of action of TA as an aphrodisiac.

In a study that looked at the knowledge, attitudes and practices related to ED, TA was recognised by Asian males as a traditional remedy in preventing or treating ED (Low *et al.*, 2002). Nevertheless, only until recently, human clinical trials demonstrating the aphrodisiac affect of TA were not available. Currently, in a 12 weeks trial by Ismail *et al.* (2012), sexual libido scores for subjects administered with a standardised aqueous extract of TA called Physta®, significantly increased between week 6 and 12 as compared to placebo ($P < 0.001$). Furthermore, significant improvements in sexual satisfaction after 12 weeks of treatment ($P = 0.001$) were recorded. Selected items in the sexual libido domain *Over the last 4 weeks, how is your interest towards sexual relationship?,* significantly increased by 14.4% from baseline for subjects in the TA group. Values of item *Over the last 4 weeks, as compared to the previous 4 weeks, the frequency of your sexual relationship is increased?*, significantly increased by 17.1%. In the same study, the overall erectile function score increased significantly from baseline to week 12 as compared to placebo ($P < 0.001$), indicating an improvement on erectile functioning in subjects using *E. longifolia* extract. The subjects on the study were healthy men with no significant problem in erectile functions.

In another study, the same standardised aqueous extract of TA (Physta®) was administered to mildly erectile dysfunctional men in a randomised, placebo-controlled trial of 26 subjects (Udani *et al.*, 2011), where significant improvements in several parameters were observed at the end of trial by week 12; Erection Hardness Scale ($P = 0.012$), Sexual Health Inventory for Males ($P = 0.03$) and Ageing Male Symptom Score ($P = 0.047$). As being one of symptoms of low testosterone levels and ageing males, ED, especially in older men, is closely related to testosterone deficiency (Köhler *et al.*, 2008; Yassin & Saad, 2008). Erectile dysfunction also manifests itself in men with already underlying health problems such as diabetes mellitus, obesity, hypertension, smoking and hypercholesterolaemia (Schulman *et al.*, 2009).

Effect of TA in Bone Health

Osteoporosis commonly plagues the aged and has been linked to testosterone deficiency in men and represents an underestimated public health problem (Kaufman *et al.*, 2000). Bone thinning as a result of hypogonadism or androgen deficiency and the resulting fractures has become one of the main causes of morbidity and mortality in men with the United States reporting 1.5 million men over 65 years old suffering from the disease (Siddiqui *et al.*, 1999).

Androgens modulate bone formation through direct androgenic activity via AR or indirect action through aromatisation to estrogens (Balasch, 2003), thereby playing an important role in regulating bone health. Bone formation is the result of testosterone being converted to, dihydrotestosterone (DHT), a potent AR activator thus stimulating osteoblast proliferation and differentiation (Vanderschueren *et al.*, 2004). Testosterone directly inhibits osteoclast formation and bone resorption (Michael *et al.*, 2005). Osteoporosis occurs when the rate of bone resorption is higher that the bone formation. To treat osteoporosis, TRT is among the the options given for androgen-deficient men (Snyder *et al.*, 1999; Aminorroaya *et al.*, 2005).

In a study by Shuid *et al.* (2011b), TA supplementation in orchidectomised male rats, an animal model for

testosterone deficient osteoporosis, significantly prevented bone calcium loss. A combination therapy of testosterone and TA reduced bone turnover and improved bone strength in orchidectomised rats when treated alone and with either one in the combination (Saadiah Abdul Razak *et al.*, 2012) The authors suggested that TA testosterone combination acted synergistically in maintaining bone turnover and strength of rats because the dosage of testosterone used in combination with TA was only half of that used in the orchidectomised animals. Shuid *et al.* (2012) further demonstrated that the possible mechanism of action of TA was the elevation of testosterone levels hence suppressing C-terminal telopeptide of type I collagen levels (CTX), a bone resorption marker, which had increased as a result of the orchidectomy of the animals. The same study revealed significantly up-regulated gene expression of osteoprotegerin (OPG), the antiresorptive decoy receptor, which counteracts the Receptor Activator of Nuclear Factor κ-B ligand (RANKL) by preventing RANKL to bind to its receptor (Teitelbaum, 2000). Thereby, OPG inhibits the osteoclastogenetic process and bone resorption. Interestingly, testosterone therapy failed to give similar reaction as TA.

Apparent contradictory results regarding the testosterone levels were obtained in the studies by Tajul Ariff *et al.* (2012) and Shuid *et al.* (2012). Both studies investigated androgen deficiency osteoporosis *in vivo*. While in the former studies, testosterone level did not increase, testosterone levels increased in the latter study. Considering that dosage, period of supplementation and the type of TA extract used were similar, the only differing factor was the age of the rats. The study by Tajul Ariff *et al.* (2012) used middle-aged rats, while the Shuid *et al.* (2012) used aged rats. The two studies show that in the absence of testes, testosterone can be produced probably via adrenal glands. More importantly, however, the treatment appeared more effective in the older animals, where low testosterone levels, as a result of ageing, are expected. The baseline serum testosterone levels appear to be lower in the orchidectomised aged rats compared with middle-aged ones. This phenomenon is in line with the purported adaptogenic effects of TA in modulating testosterone levels when supoptimal (Tambi, 2009). Increased testosterone levels may have exerted proapoptotic effects on osteoclasts, reducing the bone-resorptive activity, thus preventing bone loss (Manolagas *et al.*, 2002).

Eurycoma longifolia (El) has also been shown to increase nitric oxide production (Zakaria *et al.*, 2004) and could thus affect bones (Effendy *et al.*, 2012). Nitric oxide has been shown to promote bone formation and reduce bone resorption (Wimalawansa, 2010). Its activity is up-regulated by estradiol. As testosterone is aromatised to oestrogen (Balasch, 2003), an increase in testosterone levels might lead to increased oestrogen levels and could therefore increase NO activity, thus preventing further bone resorption and osteoporosis. Furthermore, as osteoclast activity is increased and osteoblast activity reduced during oxidative stress, the antioxidant effect of *El* could play a crucial role in scavenging free radical (Varghese *et al.*, 2013).

Effect of TA as an ergogenic herbal supplement

The ageing process is characterised by a significant decrease in muscle mass and tone and an increase in fat mass. Several studies have shown that testosterone administration decreased fat mass and increased lean body mass (Harman & Blackman, 2003; Page *et al.*, 2005). In fact, after androgen supplementation to elderly men at a dosage of 200 mg testosterone enanthate biweekly, increased muscle mass (±2 kg), arm circumference, grip strength, as well as a decrease in fat mass (Tenover, 1992, 1994) was observed indicating the anabolic effects of testosterone.

Several studies have investigated the use of TA for ergogenic benefits. Hamzah & Yusof (2003) tested 14 healthy male adults who were randomly given either 100 mg of an aqueous extract of TA or placebo and study participants performed an intensive strength training programme for 8 weeks. At the end of the study, in the group that consumed TA muscle strength increased by 6.78%, and the subjects had more lean muscle mass as compared to only 2.77% increase in muscle strength with no change in the muscle mass, in the placebo group. While the percentage of body fat reduced in both groups, the effect was more pronounced in the TA group. Muscle size as determined by the mean arm circumference, in the TA group increased significantly by 1.8 cm. Yet there was no significant change in the placebo group.

Research on the use ergogenic benefit of TA is not limited to men only. A study on 31 middle-aged women between ages of 45–59 years was carried out using a daily supplementation of 100 mg TA extract demonstrated increased muscle strength as determined by handgrip strength and bigger quadriceps muscles as determined by cross section of the rectus femoris muscle using ultrasound method when compared to the placebo group (Sarina *et al.*, 2009). On the other hand, the short-term supplementation with *E. longifolia* Jack (150 mg daily for 7 days) did not positively affect endurance in running performance (Muhamad *et al.*, 2010). In contrast, positive effects on muscle strength were observed with after 5 weeks of supplementation (Hamzah & Yusof, 2003). Similar results for short-term treatment of rats where observed by Solomon *et al.* (2013). In this study, adult male rats were treated with 200 and 800 mg kg^{-1} body

weight, respectively, for 14 days. Although sperm parameters and serum testosterone concentration improved significantly, no significant changes in lean muscle mass of the gastrocnemius mucle and the omentum fat mass were recorded. The authors conclude that the failure of TA to cause significant changes in lean muscle and omentum fat masses was due to the short treatment period. Similarly, it can be argued that low dosage and short duration of TA supplementation with the aim to improve endurance running in recreational athletes led to the failure to achieve beneficial effects (Kiew *et al.*, 2003; Muhamad *et al.*, 2010). Thus, it appears that the ergogenic effect of TA is influenced by period of supplementation and dose.

Tongkat Ali supplementation was also investigated in a pilot study in senior amateur cyclists (13 male and 12 female), aged between 57 and 72 years. A dose of 400 mg day^{-1} standardised aqueous extract of Tongkat Ali (Physta®) increased muscle strength and testosterone levels significantly compared with placebo (Henkel *et al.*, 2013). This study is also the first that investigated the effects of TA supplementation on parameters that have to be watched during TRT as they represent possible contraindications for a therapy that increases serum testosterone levels and could be indicators of muscle damage, respectively. In this study, TA treatment, which reportedly increases serum testosterone levels (Tambi & Imran, 2010; Tambi *et al.*, 2012), resulted in a significant increase in the haemoglobin concentration in men. Contrary, in women, this effect was not evident. Also, no change in the hematocrit was observed in both genders. This is an important observation as a hematocrit above 50% can trigger strokes. Creatine kinase as a parameters of muscle damage (Jones *et al.*, 1986) even decreased, though not significantly. In addition, TA treatment for 5 weeks only caused a marginal increase in the blood urea nitrogen concentration, a parameter, which under the circumstances of that study, should be regarded as indicative of proper kidney function (Kuroda *et al.*, 2012).

Effect of TA in metabolic disorder/ antihyperglycaemic/body fat

As a considerable body of evidence exists showing a link between testosterone deficiency and type-2 diabetes mellitus, insulin resistance, obesity and metabolic syndrome (Traish *et al.*, 2009; Wang *et al.*, 2011), TRT has significant beneficial effects on these conditions (Jones, 2010; Corona *et al.*, 2011b). In a meta-analysis, Corona *et al.* (2011b) showed that TRT significantly improved glycometabolic control and body mass index in diabetic men, thereby lowering the risk of early death.

Husen *et al.* (2004) reported a significant antihyperglycaemic effect of TA in a rat model where diabetes was induced by streptozotocin. In this study, four Malaysian plant extracts were administered to the animals at different concentrations. Among these four extracts, only the extracts of *E. longifolia* and *Andrographis paniculata* revealed a significant antihyperglycaemic effect. Considering that this effect was not evident in normoglycaemic subjects, this plant extract is rather 'normalising' or 'restoring' normal blood glucose levels than lowering as the testosterone levels are also thought to be 'restored' (Talbott *et al.*, 2010). Yet the molecular mechanism of this antihyperglycaemic effect has not been further investigated but might due to the increased testosterone levels.

With regard to possible beneficial effects of TA on obesity and metabolic syndrome as well as the blood lipid profile, no studies have been conducted thus far. However, as epidemiologic studies revealed that low testosterone levels affect numerous aspects of cardiovascular disease risks (Vikan *et al.*, 2009; Haring *et al.*, 2010), treatment of these patients with *E. longifolia* to increase serum testosterone levels might be beneficial as exogenous testosterone administration is associated with decreased levels of high-density lipoprotein (HDL), decreases in low-density lipoprotein (LDL) and total cholesterol (Shabsigh *et al.*, 2005; Monroe & Dobs, 2013).

The effect of *Eurycoma longifolia* on quality of life

The conventional TRT was found to improve mood and well-being and reduce fatigue and irritability in hypogonadal men (Wang *et al.*, 1996; Lunenfeld & Nieschlag, 2007). On the other hand, TA has been traditionally consumed in Southeast Asia as a health tonic (Goreja, 2004) and has recently been recognised as a traditional remedy in late-onset hypogonadism, an age-related decline in serum testosterone levels affecting quality of life in men (Tambi *et al.*, 2012) as well as an alternative treatment for idiopathic male infertility (Tambi & Imran, 2010). Proper clinical studies on the safety and efficacy of TA treatment were not established until recently (Ismail *et al.*, 2012; Tambi *et al.*, 2012).

To this date, only one large sample sized, randomised, double-blind, placebo-controlled clinical trial on the supplementation of 109 men at reproductive age with TA extract was performed (Ismail *et al.*, 2012). This study clearly showed significant improvements in all relevant parameters tested (Quality of Life as observed with the SF-36 Quality of Life questionnaire, Sexual Well-Being as investigated by means of the International Index of Erectile Function, Sexual Health Questionnaire and a semen

analysis). The physical functioning domain constituted questions on nine items on moderate and vigorous activities, climbing, bending and kneeling, walking, and bathing/dressing, 'role physical' and 'vitality'. With regards to question within Reported Health Transition Domain (*Compared to a year ago, how would you rate your health in general now?*), the group on TA achieved an overall significant change from baseline to end of study at week 12 as compared to placebo ($P = 0.009$). Furthermore, TA treatment significantly reduced fat mass in overweight subjects (≥BMI 25). All safety parameters such as blood urea serum electrolytes, alkaline phosphatase, prostate-specific antigen, lipid profile, or full blood count were comparable with the placebo group.

In another placebo-controlled trial, when 200 mg day^{-1} of aqueous TA extract was administered for 3 months to a population of 26 men with mild ED, the Ageing Male Score (AMS) score was significantly reduced in the treatment group (Udani *et al.*, 2011). Similarly, Tambi *et al.* (2012) showed a significant improvement of the AMS score in a hypogonadic population after 1 month when supplemented with the 200 mg day^{-1} aqueous extract of TA. While before the treatment 10.5% of the patients did not show any complaint based on AMS rating and 35.5% had normal testosterone levels, after the treatment, 71.7% patients reported no complaints on AMS scales and 90.8% had their testosterone levels returning to normal.

With regard to the psychological effects of TA, Ang and Cheang (1999) have demonstrated the anxiolytic effect of this herbal extract in mice. In the human, a randomised placebo-controlled study including 32 men and 32 women was conducted by Talbott *et al.* (2013). The authors showed significant improvements in moderately stressed subjects. All mood parameters, such as tension, anger and confusion, improved significantly. This is thought to be due to changes in the hormonal profile as testosterone levels increased and cortisol levels decreased leading to a significantly improved cortisol/testosterone ratio in the TA group.

Thus, TA improves quality of life by improving vitality, physical activity and a sense of general well-being, has an antiageing effect seen in the improvement of AMS, increases vigor and improves mood by alleviating anxiety, all effects that are also attributed to testosterone supplementation (Lunenfeld & Nieschlag, 2007). Taken together, based on the current findings, it appears that TA may be a safer and cheaper alternative treatment of ageing males for the negative effects of testosterone deficiency. Nevertheless, further studies have finally to confirm safety and clinical indications for such treatment. A significant advantage will be the form of administration as this herbal remedy is available in capsules.

Safety aspects of Tongkat Ali usage

Safety studies carried out thus far showed that TA concentrations used therapeutically (2.5 μg ml^{-1}) appear not to have detrimental effects on human spermatozoa *in vitro* (Erasmus *et al.*, 2012). However, at concentrations higher than 100 μg ml^{-1}, cytotoxic effects might occur (Kuo *et al.*, 2004; Nurhanan *et al.*, 2005) supporting *in vivo* data by Tambi (2006) that the extract is not toxic. In animal studies, no negative effect on the offspring could be found, neither in terms of malformations nor of any effect on body weight or the number of the offspring (Solomon *et al.*, 2013). Yet subacute toxicity tests in rats revealed an LD50 for an ethanolic and aqueous extract of TA of 2000 and 3000 mg kg^{-1} body weight, respectively (Satyavivad *et al.*, 1998; Kuo *et al.*, 2003). These authors further showed that dosages of 200 mg kg^{-1} body weight of the ethanolic extract and 300 mg kg^{-1} of the aqueous extract daily were not toxic. Only at dosages above 1200 mg kg^{-1} body weight, significant hepatotoxic effects were shown in the rat (Shuid *et al.*, 2011a).

Recently, Choudhary *et al.* (2012) investigated the acute, subacute and subchronic toxicity of the standardised aqueous *E. longifolia* extract (Physta®) in a rat model. Male and female Wistar rats were treated for 90 days with TA concentrations from 250 mg kg^{-1} body weight to 2000 mg kg^{-1} body weight. Results clearly show no significant changes in blood chemistry and haematological parameters. There were also no histopathological changes and even in acute toxicity tests, no changes in mortality or in the behaviour of the animals could be seen.

With reference to the prostate, the Endocrine Society recommends that PCa has to be regarded as a contraindication for any testosterone treatment (Bhasin *et al.*, 2010). Nevertheless, no scientific evidence for the claim that TRT is triggering or supporting PCa has been reported thus far. Considering that *E. longifolia* extract is increasing the serum testosterone concentrations, there might be a potential risk that a TA treatment of elderly men might cause prostatic problems. On the other hand, the randomised, double-blind, placebo-controlled clinical trial by Ismail *et al.* (2012) revealed no difference between the placebo and the verum group for serum PSA levels. In addition, there are indications that the aqueous extract of TA has cytotoxic activity on several cancer cell lines (Nurhanan *et al.*, 2005; Tee *et al.*, 2007; Zakaria *et al.*, 2009; Wong *et al.*, 2012; Erasmus *et al.*, 2012). If this anticancer activity of TA would be confirmed, there might be the possibility that both testosterone 'normalising' and antiproliferative activities could be combined in this natural product, providing an excellent treatment

option for ageing males' symptoms in terms of a herbal hormone replacement therapy.

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