

TONGKAT ALI PLANTS OF *EURYCOMA LONGIFOLIA* AND *STEMA TUBEROSA* STIMULATE SEXUAL AROUSAL IN DOMESTIC COCKS

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ABSTRACT This study compared the aphrodisiac ability of Tongkat Ali Merah (red type) or *Stema tuberosa* to Tongkat Ali Putih (white type) or *E. longifolia*. Fowls dosed orally with capsule containing *E. longifolia* and *S. tuberosa* respectively for 30 days followed by determining the efficacy by evaluating their sexual mating behavior parameters, testosterone blood level and histology of testicular tissue. Safety parameters included biochemical levels and histology of the liver. Both types of Tongkat Ali shown increases in testosterone levels (7.7 ± 0.59 nmol/L and 6.25 ± 0.70 nmol/L and 4.08 ± 0.85 nmol/L; *E. longifolia*, *S. tuberosa* and control fowls, respectively) as well as testicular histology showing seminiferous tubules with increased cellularity with no evidence of inflammation or fibrosis compared to the control to indicate as testosterone boosters. All biochemical parameters tested shown to be within the control values except for alanine aminotransferase of *E. longifolia* i.e. 23.50 ± 6.36 u/L (control = 8.00 ± 2.94 u/L). Additionally, some moderate tissue changes (focal areas of congestion of central vein and periportal scattered inflammation) were visible for the liver taken from *E. longifolia* treated fowls. *S. tuberosa* found without any untoward effects. Tongkat Ali Merah hereby been confirmed to boost testosterone in fowls although not as effective as Tongkat Ali Putih.

ABSTRAK Kajian ini memperbandingkan keupayaan afrodisiak Tongkat Ali Merah atau *Stema tuberosa* dengan Tongkat Ali Putih atau *Eurycoma longifolia*. Uggas disuap dengan kapsul yang mengandungi *E. longifolia* dan *S. tuberosa* selama 30 hari bagi mengetahui keberkesanannya dengan menguji tingkah laku seksual mereka, paras darah testosteron dan histologi tisu testis. Parameter keselamatan yang diuji termasuk tahap biokimia dan histologi hati. Kedua-dua jenis Tongkat Ali menunjukkan kenaikan paras testosteron (7.7 ± 0.59 nmol / L dan 6.25 ± 0.70 nmol / L dan 4.08 ± 0.85 nmol / L; bagi *E. longifolia*, *S. tuberosa* serta ungahan kawalan, dalam urutan yang diberikan dan juga histologi testikular yang menunjukkan tubulus seminiferous dengan peningkatan selular tanpa apa-apa kesan keradangan atau fibrosis berbanding dengan kawalan iaitu membuktikan peningkatkan pengeluaran testosteron pada sel berkenaan. Semua parameter biokimia yang diuji menunjukkan pada persekitaran nilai kawalan yang juga diuji kecuali alanine aminotransferase bagi *E.*

longifolia iaitu dengan peningkatan ketara, 23.50 ± 6.36 u / L (kawalan = 8.00 ± 2.94 u / L). Selain itu, ada beberapa perubahan yang tidak diingini kepada tisu hati tetapi adalah dengan sederhana kesannya (kawasan tumpuan kesesakan vena pusat dan keradangan bertaburan periportal) bagi unggas dirawat dengan *E. longifolia*. *S. tuberosa* pula didapati tiada sebarang kesan yang tidak diingini sama sekali. Tongkat Ali Merah dengan ini disahkan dapat juga meningkatkan paras testosterone dalam unggas walaupun tidaklah setanding seperti Tongkat Ali Putih.

Keywords: Testosterone, Libido, Herbal, Sex, Chicken

1. INTRODUCTION

Tongkat Ali is known worldwide as an aphrodisiac plant. Not known to many is that the name is commonly applied to at least three types of plants in Malaysia i.e. *Eurycoma longifolia*, *Stema tuberosa* and *Polyalthia bullata*. The keyword, Tongkat Ali, is extremely popular with thousands of products to appear in the internet search if attempted. Many of the products available in the worldwide market been described merely as Tongkat Ali either at the product brand or their ingredient. Sales of the products are lucrative and mostly done online. Nowadays, western world is increasingly reliant on herbal medication (Garcia-Alvarez et al., 2014; Smith et al., 2015). Previously some doubts of the authenticity of the

Tongkat Ali products have already been highlighted whereby almost half of the products tested for two markers were found negative for the presence of the markers (Vejayan et al., 2018).

There are at least five plants using the synonym Tongkat Ali with three most common ones; *E longifolia*, *S. tuberosa* and *P. bullata*. These plants are pronounced as Tongkat Ali by indigenous and local Malay people in Malaysia with each distinctly known as Tongkat Ali Putih (white), Tongkat Ali Merah (red) and Tongkat Ali Hitam (black), respectively based on the colour of their root (Figure 1) (Kuo et al., 2004). The most popular of the three Tongkat Ali is *Eurycoma longilofia* Jack, commonly been used as herbal medicine worldwide (Mahmoud and Noor, 2013).



Figure 1. The cross section of roots from (a) *E. longifolia* (Tongkat Ali Putih), (b) *S. tuberosa* (Tongkat Ali Merah) and (c) *P. bullata* (Tongkat Ali Hitam). Insert: a ruler shown measurement in inches.

The aphrodisiac properties of *E. longifolia* have been extensively studied *in vitro*, *in vivo* and clinically (Ang et al., 2000; Bhat and Karim, 2010; Mohamed et al., 2015; Rehman et

al., 2016; Scarano et al., 2006; Tambi et al., 2012). *E. longifolia* become a popular herbal medicine in Asia due to its high aphrodisiac properties. Many products have been developed based on

E. longifolia and commercialized either in the capsule form, chips form or in the pre-mix coffee. Malaysian plants such as *E. longifolia*, *S. tuberosa* and *P. bullata* are claimed to have aphrodisiac properties and have been used in Malay traditional medicine. The aphrodisiac property of these plants is only a claim and has not been scientifically proven except for *E. longifolia* (Ang et al., 2000; Bhat and Karim, 2010; Mohamed et al., 2015; Rehman et al., 2016; Scarano et al., 2006; Tambi et al., 2012).

S. tuberosa is currently experiencing some discrepancies related to its scientific name. It is sometimes mentioned as *Jackia ornata* or *Jackiopsis ornata* (Family: Rubiaceae) (Abugabr Elhag et al., 2019). In this report the red variety root Tongkat Ali will be referred simply as *Stema tuberosa*. Among the three types, *S. tuberosa* or Tongkat Ali Merah has been claimed to be the most sorted Tongkat Ali by revisiting customers. Unfortunately, no scientific information was available on this plant.

In considering the availability of more than one type of plants under the synonym of Tongkat Ali to be used in manufacturing of aphrodisiac products ideally a study to compare efficacy and safety of *E. longifolia* and *S. tuberosa* been carried out.

2. MATERIAL AND METHODS

2.1 Animals selection

The male chicken or roosters used in this study were healthy Red Jungle Fowl (*Gallus gallus*). A total of 12 fowls used with four for each 3 groups (control, *E. longifolia* and *S. tuberosa*). All the roosters selected

were aged between 26 - 28 weeks before their fertility peaks which was between 30 and 40 weeks of age. The average weight of fowls initially was about 1.25-2.0 kg. Animal ethics approval obtained UMPIACUC/2018/01.

2.2 Capsule preparation

Tongkat Ali roots obtained from reliable indigenous people living in the village of Kampung Orang Asli Bukit Cermin, Perak, Malaysia (GPS: 4°45'48.2"N 100°58'13.3"E). Once the quality of *E. longifolia* was authenticated for the existence of eurycomanone (chemical marker) as well as that of protein Marker A as described by Vejayan et al. (2018), a conventional basic capsule marker was used to formulate the capsule. The "0" sized capsules were filled with pulverised and sieved (sieve pore size: 200 mesh) root chips without additives or excipient. Similarly, *S. tuberosa* root powder filled capsules were prepared. The dosage of *E. longifolia* and *S. tuberosa* used was 12 mg (calculated based on the average weight of the chicken) per capsule. Any empty space within the capsule were occupied with powdered chicken feed (corn bran).

2.3 Dosage method

The control (capsule containing chicken feed only), *E. longifolia* and *S. tuberosa* groups of fowl (4 each) were given one capsule in the morning and another in the evening ("bis in die", b.d.) daily for 30 days. The orogastric procedure involved placing the capsule into a forced wide open beak followed immediately by supplying with a straw small quantity of drinking water and consequently shutting tightly the beak. Momentarily the chicken was noticed to swallow willingly the capsule. An inspection of the mouth cavity done

after sometime to ensure dosing completed successfully.

2.4 Sexual mating behaviour

There a few indicators that will be used as parameters in the observation of sexual mating i.e. frequency of wing flapping, body-shake, crow and pecking (Leonard and Zanette, 1998). A rooster was placed for an hour with healthy hen age about a month after 30 days of dosing to record changes in mating behaviours and weight (initial, days 15 and 30).

2.5 Blood collection

After evaluating the roosters' sexual mating behaviour, the blood drawn from control and test subjects. A total of 5 ml of blood collected from the rooster by slaughtering. The blood that drawn out after slaughtering collected by using a beaker. A syringe used to draw the blood collected and transferred into a vacutainer (obtained from Gribbles Pathology Diagnostic Laboratory, Malaysia). The blood samples collected and send to Gribbles Pathology Diagnostic Laboratory, Malaysia and tested on various biochemical parameters.

2.6 Histology on testis and liver

Testis and liver dissected out carefully and placed onto 5ml of 10% formalin overnight. The preparation and grading of the tissues are as done by Haleagrahara et al, 2009 and 2010. Briefly the tissues were sliced, and exposed to graded xylene, alcohol and then embedded in paraffin wax blocks.

Sections are taken at 5 microns using a Leica microtome on glass slides and stained with hematoxylin and eosin. The prepared slide analyzed under a light microscope (*Nikon, Eclipse TS100*) for inflammation, congestion, and grading for all the groups.

2.7 Statistical analysis

Data collected for the mating observations and biochemical parameters were evaluated by ANOVA from the Social Science (SPSS) version 20. Statistical significance of data was assessed by analysis of variance and differences were considered significant at ($p<0.05$). The least significance difference (LSD) test were used to compare the means.

3. RESULTS AND DISCUSSIONS

3.1 Monitoring of body weight

The fowls were weighed initially and on the 15th and 30th days. Over the 30th day observations none of the four fowl in any group required to be excluded or replaced due to drastic weight lost. Instead, the results in Table 1 showed weight gains on the 15 day for all groups while only *E. longifolia* fowls showed weight gains beyond the 15 days. Previously it has been reported *E. longifolia* treated rats capable to have their laevator ani muscle increased in weight after dosing for 12 consecutive weeks (Ang and Cheang, 2001). While in men, *E. longifolia* increased the fat free mass, reduced body fat, and increased muscle strength and size (Hamzah and Yusof, 2003).

Table 1. Weight of fowls during experiment

Fowls	Initial average weight (kg) ± S.D	Gain (gm) ± S.D in weight to initial		End average weight (kg) ± S.D
		Day 15	Day 30	
Control	2.025 ± 0.22	75 ± 9.5	0 ± 0.0	2.1± 0.30
<i>E. longifolia</i>	1.35 ± 0.19	50 ± 2.5	125.5 ± 2.3	1.53± 0.27
<i>S. tuberosa</i>	1.6 ± 0.17	50 ± 1.7	0 ± 0.0	1.65± 0.16

Result showed the weight gain by the fowls during experiment, which n=4 fowls for each group and mean ± standard deviation

3.2 Sexual mating behaviour observation

Table 2 compared the frequencies in the sexual mating behaviours of the fowls after being treated with *E. longifolia* and *S. tuberosa*. *E. longifolia* showed

increases of sexual mating frequency compared to *S. tuberosa* and with both plants being higher than the control fowls for all type of behaviours. Overall, the results showed that *E. longifolia* give the highest frequency for all sexual mating behaviour.

Table 2. Summary of the sexual behaviour frequency observed

Types of Sexual Behaviour	Sexual Frequency Observed		
	Control	<i>E. longifolia</i>	<i>S. tuberosa</i>
Wing Flapping	4.0 ± 0.4	6.0 ± 0.5	5.5 ± 0.2
Body-shakes	4.3 ± 0.2	7.3 ± 0.4	5.5 ± 0.3
Crows	3.5 ± 0.3	6.0 ± 0.6	5.2 ± 0.4
Pecking	3.0 ± 0.0	7.0 ± 0.6	4.5 ± 0.3

Results were expressed as mean ± SEM. The level of significance was taken at p<0.05 compared with the control, n=4.

3.3 Blood plasma level

As shown in Table 3, testosterone for the *E. longifolia*, 7.70+0.59 nmol/L found almost twice the value of the control, 4.07 ± 0.85 nmol/L. Although not to the extent of *E. longifolia*, *S. tuberosa* also showed an elevation of testosterone level with no obvious increment of any of the biochemical parameters tested. Hence through this results *S. tuberosa* provided evidence of stimulating testosterone hormone within 30 days of dosing without any untoward circumstances. The capability of *E. longifolia* in stimulating this androgen hormone is well documented (Talbott,

2019). In some study on human, *E. longifolia* has been found to increase the serum testosterone level, treat erectile function disorder and improve sperm production problems (Hamdi et al., 2016). Besides that, most studies used rat to test *E. longifolia* as it is able to increase libido in male rats and decrease hesitation time for male rats toward female rats (Ho and Tan, 2011).

All the other biochemical parameters provided only slight reduction or elevation except for the ALT (alanine aminotransferase) indicators of *E. longifolia*. The value observed for the biochemical parameter of the ALT for *E. longifolia* of 23.50 ±

6.36 u/L was markedly elevated, almost three-fold increase in comparison to the control value of only 8.00 ± 2.94 u/L. In human, ALT and AST (aspartate aminotransferase) are two indicators of liver damage with the former to be more specific to liver. Based on American Association for the Study of Liver Diseases (AASLD), ALT levels greater than 5 times the upper limit of the normal range suggests a potentially serious, active liver disease process (Kim et al, 2008). Therefore, result shown by *E. longifolia* treated fowls having approximately 3 times more ALT levels than the control may

suggest potential liver damage however can only be ascertained clearly if investigated further for prolong or chronic use of more than 6 months. Interestingly, sub-chronic studies on rats treated with *E. longifolia* concentrations of up to 2000 mg/kg bodyweight for a period of 90 days shown have no significant blood chemistry (including ALT) and haematological parameters (Choudhary et al., 2012). Choudhary et al, treated the rats orally by gavage an aqueous extract *E. longifolia* once a day for 90 days.

Table 3. Biochemical parameters for both *E. longifolia* and *S. tuberosa*

Biochemical parameters	Control	<i>E. longifolia</i>	<i>S. tuberosa</i>
Testosterone (nmol/L)	4.08 ± 0.85	7.70 ± 0.59	6.25 ± 0.70
ALT (u/L)	8.00 ± 2.94	23.50 ± 6.36	5.25 ± 0.5
AST (u/L)	312.67 ± 49.80	308.25 ± 55.95	232.75 ± 21.58
GGT (u/L)	22.00 ± 3.51	18.75 ± 10.34	23.50 ± 4.20
Total Protein (g/L)	50.75 ± 2.99	45.25 ± 4.86	40.75 ± 3.86
Albumin/Globulin ratio (g/L)	0.53 ± 0.05	0.63 ± 0.17	0.65 ± 0.13
Total Bilirubin (umol/L)	50.75 ± 2.99	47.25 ± 2.5	17.75 ± 1.5
Urea (mmol/L)	1.03 ± 0.26	1.10 ± 0.36	1.28 ± 0.35
Creatinine (umol/l)	12.75 ± 0.96	10.00 ± 1.41	17.50 ± 6.45
Uric Acid (mmol/L)	0.12 ± 0.03	0.13 ± 0.01	0.18 ± 0.025

Data were expressed as mean \pm SEM. The level of significance was taken at $p < 0.05$

3.4 Histological profile of liver and testis

The prepared slides were analyzed under a light microscope (Nikon, Eclipse TS100) for inflammation, congestion, and grading for all the groups. Photomicrographs were taken by Nikon 8.1 MP camera using Nikon Eclipse software.

Table 4 showed moderate changes on congestion of liver for EL2-EL4 and with only mild changes for inflammation or necrosis of liver in comparison to controls. As for ST1-ST4, the changes in liver were similar to EL group, with focal areas of necrosis and regeneration scattered through the parenchyma. Mild to moderate congestion was seen in all livers.

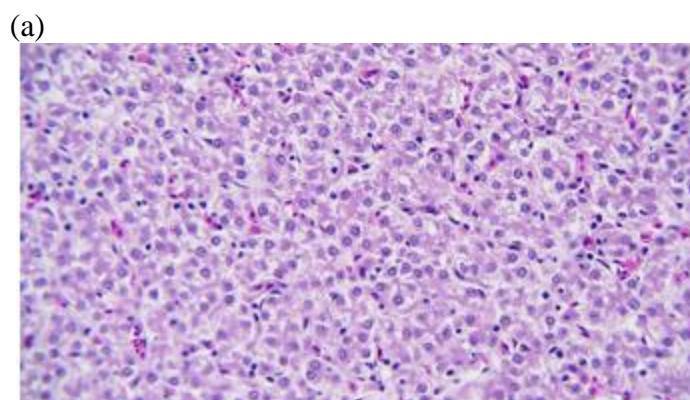
Table 4. Histopathological evaluations of liver treated with *E. longifolia* and *S. tuberosa*

Fowls	Inflammation	Necrosis	Congestion
EL1	0	0	0
EL2	+	0	++
EL3	+	+	++
EL4	+	0	++
ST1	+	+	+
ST2	+	+	++
ST3	+	0	+
ST4	+	+	++

Keys: EL & ST – rooster test fowls given *E. longifolia* and *S. tuberosa* containing capsules for 30 days. The histological scoring scale used was 0 = no changes, + = mild, ++ = moderate, +++ = severe compared to control fowls.

As shown in Figure 2(a), for control rooster the liver showed normal architecture with normal central vein and well-spaced portal triads, no evidence of congestion. Hepatocytes and sinusoids appeared normal. While on Figure 2(b-c), EL4 and ST4, showed focal areas of congestion of central vein and periportal scattered inflammation, suggesting that perhaps the dosage administered was causing hepatic changes suggestive of drug metabolism, but not significant enough to cause necrosis or damage.

In summary, the liver tissues taken from fowls treated with *E. longifolia* provided outcome which was not severe based on its histopathological results alone. Nevertheless, in combining this results with the obvious elevation of ALT (Table 3) the severity to liver is questionable if the study been carried forward for a longer duration (i.e. 6-12 months) for *E. longifolia*. No such concerns existed for *S. tuberosa* as it provided positive results for histopathological liver grading and its corresponding biochemical markers (i.e. ALT, AST and GGT).



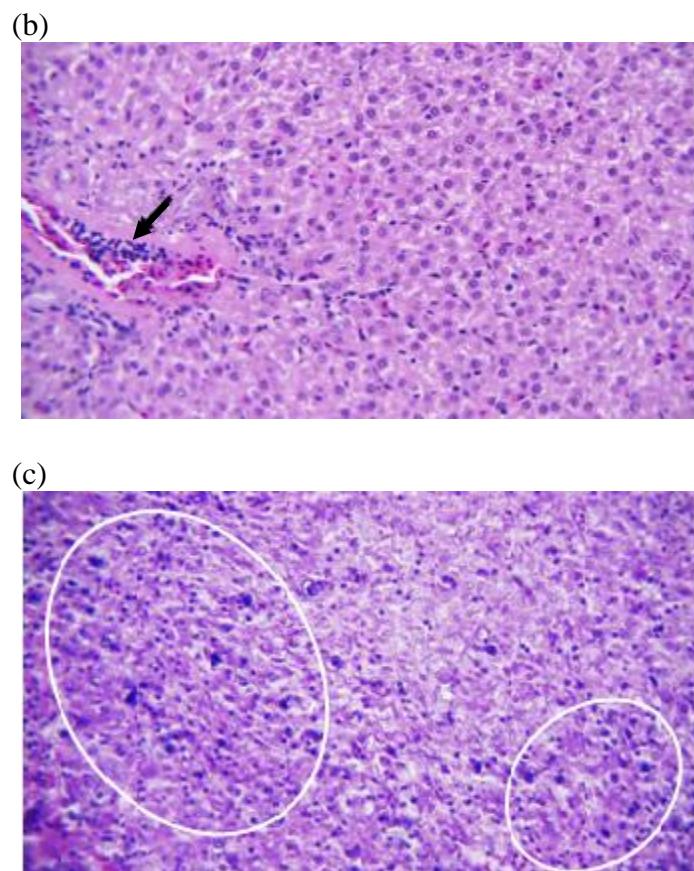


Figure 2: Photomicrograph of liver tissue, H&E, 400X. Histology of liver showing normal (a), EL4 (b), showing periportal inflammation (arrow) and scattered diffuse inflammation and ST4 (c) group of fowls showing diffuse inflammation and areas of regenerative hepatocytes (circles).

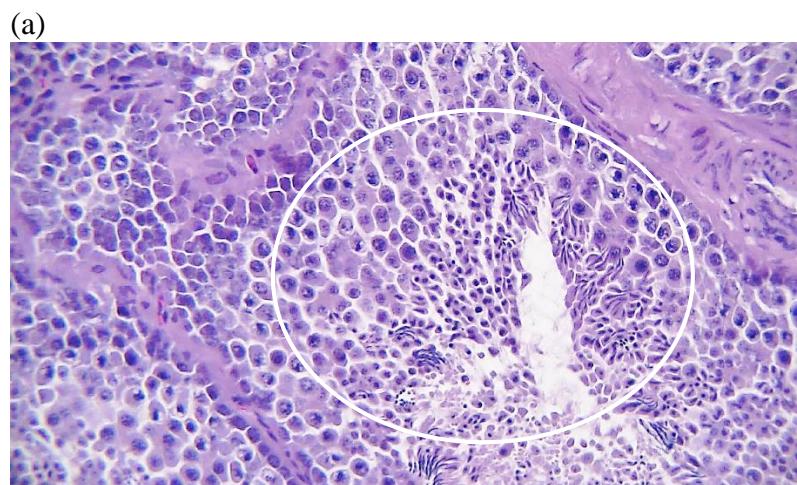
Plants have been generally regarded as safe compared to synthetically sourced constituents as drugs (Di Lorenzo et al., 2015; Posadzki et al., 2013). Though that is the general understanding of herbal consumption but the wellbeing of liver has been of concern in recent years (Seeff, et al., 2015). *E. longifolia* safe consumption is questionable based on the elevation of ALT biochemical indicator and with moderate outcome for the liver histological grading. Shuid et al, 2011 (Shuid, et al., 2011) reported that the sub-acute oral intake (28 days) of 2400 mg kg^{-1} *E. longifolia* aqueous extract was hepatotoxic to rats. It is easily possible to provide reasoning for this safety concerns to be minor and

negligible but as it is common for any herbal capsule to be taken twice a day continuously some caution is warranted for deterring liver failure. Personal communications with Malaysian indigenous people in Kampung Orang Asli Kampung Cermin described some of their long term customers decided to only consume one capsule once a day after some years. Such dosing regimen should also apply in elderly or patients with liver problems. *E. longifolia* use at low doses does not appear to cause any toxic effect on the pancreas over a period of more than a month (Hamoud and Qamar, 2013). *E. longifolia* is normally recommended to be administered to men at the dose of 200–400 mg daily (Shuid, et al., 2011).

As shown in Figure 3(a), a segment of the testis showed numerous seminiferous tubules, sized at approximately 150-200 microns, showing adequate cellularity of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. There are scattered leydig cells. No evidence of inflammation or fibrosis. While on Figure 3(b-c), the testis of EL4 and ST4 fowls showed increased seminiferous tubules, sized at approximately 200-250 microns, which were larger than the control group. The tubules showed increased cellularity compared to the control, with increase in all cells - spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The spermatogonia were prominent in the centre of the lumen with long tails and closely clustered together. No evidence of inflammation or fibrosis. This suggest

that *E. longifolia* and *S. tuberosa* had direct stimulating effects on both the seminiferous tubules, and also the mitosis of the germ cells and also the maturation of the cells.

Hence overall *E. longifolia* shown as expected of its testosterone boosting capabilities similar to previous studies (Ang and Lee, 2002; Ang and Ngai, 2012; Tambi et al., 2012; Zanolli et al., 2012). Additionally, positive evaluations were attained of the mean frequency sexual mating behaviours and histology on testis of test fowls. Coincidentally histology of testicular tissues obtained from rats treated for 28 days with *E. longifolia* roots showed active germinal epithelium with successive stages of spermatogenesis and lumen of the seminiferous tubules were filled with spermatozoa in comparison to control (Mahmoud and Noor, 2013).



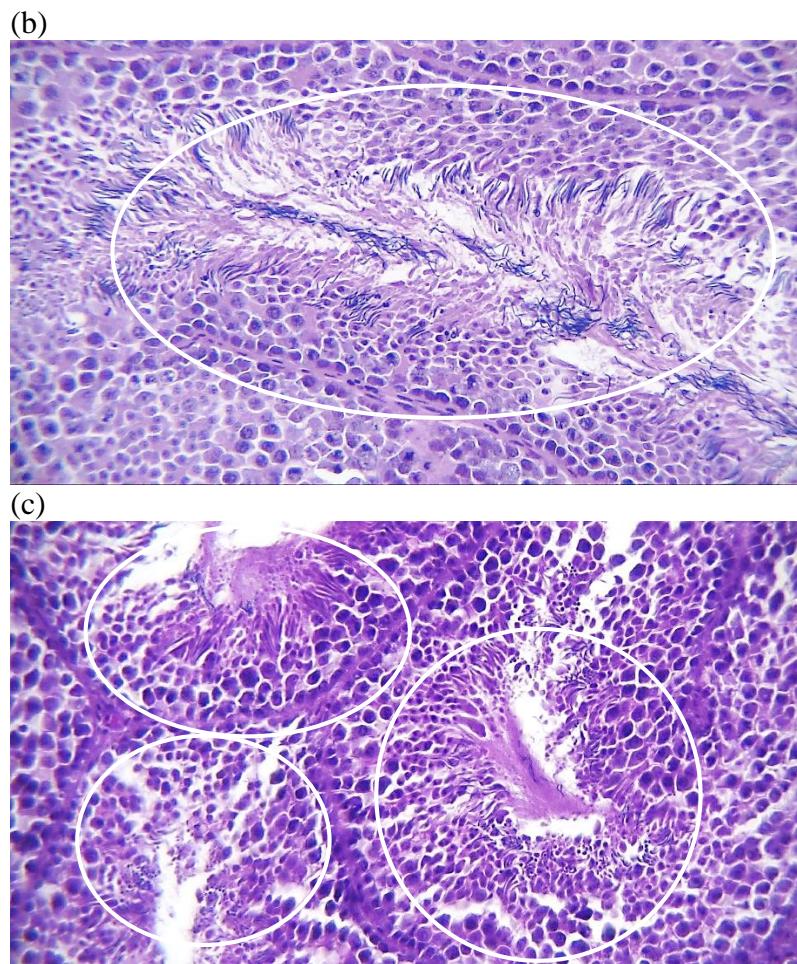


Figure 3. Photomicrograph of testis tissue, H&E, 400X. Histology of testis showing normal (a) showing adequate proliferative activity of the cells, EL4 (b) and (c) ST4 group of fowls showing increased proliferation (both number and maturity) of all the types of cells in the seminiferous tubules including mature spermatozoa in EL4 and ST4 groups, as in circles.

S. tuberosa, although not as potent an aphrodisiac as *E. longifolia*, showed sufficient results to substantiate its claim as being a testosterone booster. *E. longifolia* treated fowls shown an increase of approximately 90% testosterone concentration while *S. tuberosa* with approximately 55% increase in comparison to the untreated fowls i.e. 4.08 nmol/L. Histology on the testis of test fowls dosed with *S. tuberosa* provided further evidence on the stimulation of specific region within the male reproductive organ. The total frequencies tabulated for the sexual mating behaviours (wing flapping, body-shakes, crows and pecking) for *S.*

tuberosa (approximately 21 counts) were less convincing compared to *E. longifolia* (approximately 26 counts) but both were higher than control (approximately 15 counts). In the short duration of dosing been done, i.e. 30 days, both plants performed well as sexual stimulant with *E. longifolia* ranked above *S. tuberosa*.

4. CONCLUSIONS

Although *E. longifolia* showed higher efficacy indicators i.e. sexual mating behaviours, testosterone level and active testicular histology it is

however marred by its safety related to elevate ALT and moderate damage to the liver as observed within 30 days of exposure. On the other hand, *S. tuberosa* is safer as compared to *E. longifolia* but it exhibited lesser aphrodisiac activity.

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