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Research Article

Evaluating the estrogenic activity and toxicity of *Tectona grandis* leaf extract on the reproductive and endocrine system of female wistar rats

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Abstract

Female reproductive complications continue to be a major health challenge worldwide. Medicinal plants richer in secondary metabolites such as phytoestrogens have been used over decades for the treatment of reproductive health problems like infertility, with limited knowledge on their toxicities. The present study was designed to evaluate the estrogenic potential and safety of *Tectona grandis* leaves extract on female wistar rats.

Following acclimatization and pre-evaluation of the estrous cycle, female wistar rats, 6 weeks old were placed in groups of 3 animals each and *T. grandis* extracts administered daily in graded doses of 500, 1000, 2000mg/Kg body weight against controls for 28 days (Sub-acute toxicity). A dose dependent increase in 17-Beta estradiol was observed in the serum and ovary homogenates versus an increase in cholesterol when compared to the control groups. Results from the three animals per group showed an increase in the weights of the animals and a non-significant increase in alanine aminotransferase (ALT), white blood cells, haemoglobin and haematocrit at the dose of 2000 mg/kg. Also, there was no significant difference in the organ weights and histopathological examinations of necropsied animals showed no abnormalities in the various organs. *T. grandis* leave extract contains phytochemicals such as lignans that can be converted by microflora to phytoestrogens, which can compete with endogenous estrogen for the estrogen receptor potentiating similar activities like estrogens. This indicates that *T. grandis* could be explored as hormonal replacement therapies in infertility, menopausal and/or breast cancer related problems.

Keywords: 17-β-estradiol, *Tectona grandis*, lignans, phytoestrogens, toxicity, hormonal replacement therapy

INTRODUCTION

Traditionally, rural women have used phytotherapy rather than modern medicine for their personal ailments due to lack of modern facilities nearby in their regions and limited funds available for their healthcare. Many *in vivo* studies have been performed by using crude plant extract or secondary metabolites on the regulation of reproductive function (folliculogenesis and steroidogenesis) [1,2,3,4,5] by mimicking the biological effects of endogenous hormones and binding to their nuclear receptor or regulating the metabolism of key enzymes [6]. Therapeutically, species of the *Tectona genus* are very important, as *T. grandis* leaves reputed to have depurative, diuretic, stimulant, anti-dysenteric and vermifuge properties. In traditional medicine, its leaves are used to treat anemia, asthenia, fevers, malaria, amoebiasis, bilharzia and tuberculosis [7]. In Cameroon, *T. grandis* is known for its laxative properties in Adamaoua (Banyo) and is also used in the South-west to treat fever. The oil extracted from young shoots is used to treat scabies. The flowers of this tree are diuretic and used to treat bilious affections, bronchitis and urinary diseases. Species of the *T. genus* are ornamental trees, usually planted in towns and along roadsides and the leaves are commonly used for food packaging in markets by indigenous populations [7]. Previous phytochemical studies on *T. grandis* have led to the isolation of naphthoquinones, anthraquinones, terpenoids [8,9,10,11], apocarotenoids [10] and norlignans [11]. Some medicinal plants are rich in compounds which have the regulatory potential effect on reproductive system by exerting their actions directly or indirectly on the hypothalamic–pituitary–ovarian axis by induction or inhibition of ovulation and steroidogenesis [1]. Oestrogens are key hormones responsible for the progression and regulation of female and male reproductive system. They play an important role in the non-reproductive system, like growth and differentiation and have a

variety of pharmacological functions such as the maintenance of bone mass, cardiovascular protection and brain protection [12]. Moreover, menopause in women is characterized by a deficiency of estrogens, leading to the risk of many health problems including hot flushes, sleeping disorders, vaginal dryness, joint pain, mood swings, reduced bone density and cardio vascular diseases [13]. The cardinal symptoms of menopause are due to the loss of ovarian oestrogens production urging the use of menopausal hormone therapy (MHT) that includes oestrogen or an estrogenic compound while preventing menopause-associated bone loss and cardiometabolic changes. Therefore, our study seeks to investigate beneficial implication of natural compounds from *T. grandis* on the female reproduction, while assessing their toxicity for the development of new hormonal therapies.

METHODOLOGY

Botanical description of the genus *Tectona*

T. grandis Linn, also known as teak, is a slow-growing or fast-growing tree, depending on climatic conditions. Its trunk is straight and cylindrical and can reach a diameter of 1.5 m for a height of 40 to 50 m and branchless up to 20 m [14].

Plant Material

The leaves of *T. grandis* were collected in the Limbe environ, South-West Region, Cameroon in August 2009. The sample identification was performed at the Cameroon National Herbarium, Yaoundé, where a voucher specimen was deposited (Voucher No. 61993 HNC)(Figure 1).

Extraction and isolation

The leaves of *T. grandis* were dried in an oven at 40˚C. The obtained dried leaves *T. grandis* (3 kg) were extracted with ethanol (25 L) for 72 h at room temperature. The ethanol filtrate was concentrated using a rotary evaporator and the yielded crude extract (120 g) kept at 4˚C in the dark until usage.

Figure 1: Picture of *T. grandis* Linn leaves [8]

Experimental Animals and Ethical consideration: Wistar albino rats were used in this study. They were bred in the animal house of the Laboratory of metabolic studies, Institute of Medical Research and Medicinal plants studies, Yaoundé, Cameroon. The rats were housed in groups of three (3) in metallic cages at 24±1°C in a 12h: 12h dark: light cycle. The animals were provided with a standard diet and water *ad libitum*, and the food was withdrawn 6h before the start of the experiment and each time before dosing. This work was carried out with respect to the welfare of animals, and the ethical clearance Nº: BTC-JIRB2024-098 was obtained from the Joint Institutional Review Board for Animal and Human Bioethics (JIRB) of the University of Yaoundé I, Cameroon. Ethical guidelines and procedures for handling experimental animals were respected.

Study Duration: Following acclimatization and pre-evaluation of the estrous cycle, the female performance was assessed approximately 28 days. A 28-day sub-acute toxicity study was conducted according to the standard methods [15,16] with fifteen (15) females; (age: 6 weeks; mass: 50 - 71g) randomly assigned to 5 groups of 3 rats per group.

Extract administration: The vehicle was an aqueous mixture of 10% ethanol prepared in water given that the extract doesn't readily dissolve in water. The rats were administered the aqueous extract of *T. grandis* at 500, 1000 and 2000mg/kg body weight (BW), against a normal control (H_2O only) doses per body weight for 28 days (previous acute toxicity studies by Kamsu *et al.* [17] showed that the lethal dose (LD50) of this extract is greater than 5000mg/kg BW. On the 28th day of the test, after gavages, the animals were subjected to a 12-hour food fast at the end of which they were anesthetized with ether soaked in cotton, and the blood was collected by cardiac puncture into EDTA and dry tubes.

Assessment of the estrous cycle: The estrous cycle which refers to the reproductive cycle in rodents was assessed based on four phases, namely proestrus, estrus, metestrus and diestrus which lasts for 4 to 5 days [18]. The vagina has different appearances at different phases of the oestrous cycle [19].

Proestrous: Vagina is gaping and the tissues are moist and reddish pink. There are numerous longitudinal folds or striations visible on the dorsal and ventral lips (lasts for 14 hours).

Estrous: Vaginal appears similar to that seen at proestrus, but the tissues are lighter pink and less moist. The striations are more prominent (24-48 hours).

Metestrus: Vagina tissues are pale and dry. The dorsal lip is not as oedematous as in the estrus/ Whitish cellular debris may line the inner walls or partially fill the vagina (6-8 hours).

Diestrus: Vagina is moist and has a small opening and the tissues are bluish-purple in color (48-72 hours)

Histopathological Analysis:

Histopathological analysis was performed on liver, spleen, heart, uterus, ovaries and kidney tissue sections after fixing in 10% formalin in distilled water. Sections of 5µm were obtained on a rotary microtome, and then, the material was stained by hematoxylin-eosin (HE). The stained slides of the sections of the tested animals

were then analyzed using an inverted microscope with an integrated digital photo camera under an objective magnification of 40X to check for anomalies. The histology of the treated groups was compared to the histology of the control group. After examination, photomicrographs of the organs were selected and they represent the general appearance observed in three animals per group.

Hematological Analysis. After the puncture, a small amount of blood (1mL) was then introduced into a sterile tube containing an anticoagulant (EDTA) for blood count analysis and was immediately used to perform a blood count using a Sysmex hematology automated analyzer.

Blood chemistry Analysis. Blood samples collected were centrifuged at 3000×g for 10min at 4°C. The serum was separated from the blood after centrifugation and stored at -80°C until analysis. Biochemical parameters like total protein, creatinine, uric acid, total cholesterol, glucose and alkaline phosphatase (ALP) (Biolabo Kits, France), were performed using commercial kits following manufacturer's instructions.

Liver fucnction enzymes: Aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured spectrophotometrically using commercial kits following the manufacturer's instructions (Biolabo, France)

Hormonal analysis: Rat 17-β-estradiol (E2) was measured in the serum of the various animals using a pre-coated competitive- ELISA detection Kit (Biomatik, EKF57964) according to the manufacturer's instructions. The absorbances were read using an ELISA plate reader (Biobase) at a wavelength of 450nm. The E2 concentrations were obtained from the standard curve equation (range: 12.5-800pg/mL, sensitivity: 7.5pg/mL).

UPLC-ESI/MS analysis of *T. grandis*

High-resolution mass spectra were obtained with an QTOF Spectrometer (Bruker, Germany) equipped with a HESI source. The spectrometer was operated in negative mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.40 ppm deviation using Na Formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) UHPLC system consisting of LC-pump, Diode Array Detector (DAD) (λ: 190-600 nm), auto sampler (injection volume 10 µL) and column oven (35 °C). The separations were performed using a Synergi MAX-RP 100A (50x2 mm, 2.5µ particle size) with a H_2O (+0.1 % HCOOH) (A) / Acetonitrile (+0.1 % HCOOH) (B) gradient (flow rate 500 µL/min, injection volume 5 µl). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min. *T. grandis* leaves extract was diluted in HPLC grade methanol (5 mg/mL) and filtered. 5µL aliquot was injected.

UPLC-ESI/MS data analysis and compounds annotation

Raw data files from the Bruker spectrometer MS (.d) were converted to a format compatible with our analysis software (.raw to .mzML). Spectral data (.mzML files). The converted data were processed with MZmine 3.4.16 [20], using the following workflow: retention time window: 0-12 min; noise level: 0; maximum RT shift was 0.01 min, and maximum mass tolerance 5 ppm for isotopes grouping. Annotation of the obtained feature sets was processed by the NIST MS search and chemical formula prediction options of the Mzmine console. A minimum cosine of 0.7; m/z tolerance of 10 ppm and the following adducts $[M - H]^-$, $[M - Cl]^-$, were used for formula prediction.

Statistical Analysis: All variables were subjected to descriptive data analysis. All continuous variables were expressed as the mean and the standard error of the mean. GraphPad Prism version 8.0.2 was used. The results were analyzed statistically using one-way ANOVA and Dunnett's multiple comparison test to identify the differences between treated groups and controls. The data was considered significant at $p < 0.05$. The correlation coefficient was calculated between total cholesterol and estradiol (serum and ovaries) at $p < 0.05$.

RESULTS

Effect of extract on animal weights and general behaviour

The extract did not influence significant changes in body weight. However, the percentage weights of the animals increased proportionally with the duration of the study (Figure 2). Equally, no significant change was observed in the behavioral pattern of the treated rats, such as trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, agitation/aggressiveness, sensitivity to pain/noise, mobility, hair loss, sleep, lethargy, restlessness, or in physical appearance such as eye color, mucous membrane, skin/fur effects, body weight, injury, when compared to the control.

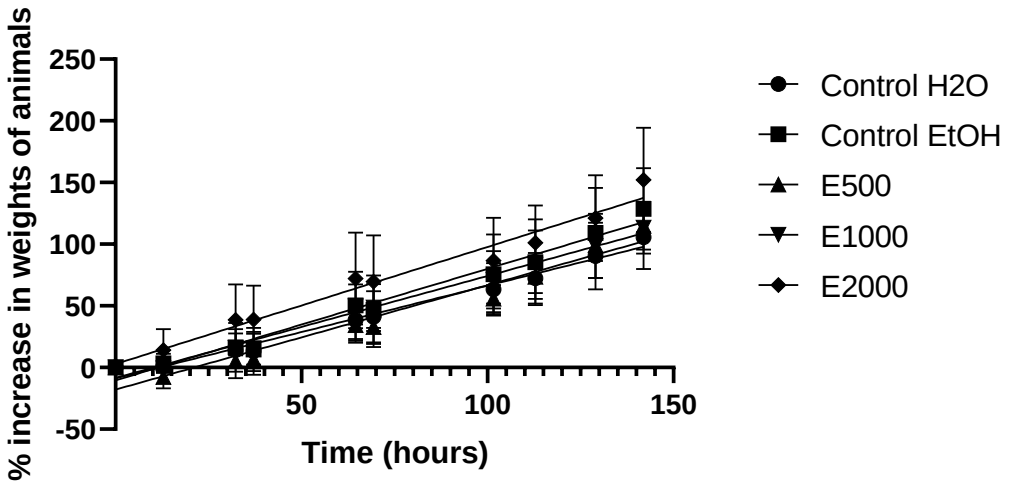

Figure 2: Percentage increase in the weights of animals

Also, after 28 days there was no significant increase in the weight of the visceral organs (liver, kidney, heart, ovaries and spleen) of the test animals which received ethanol extracts when compared to the control groups (Figure 3).

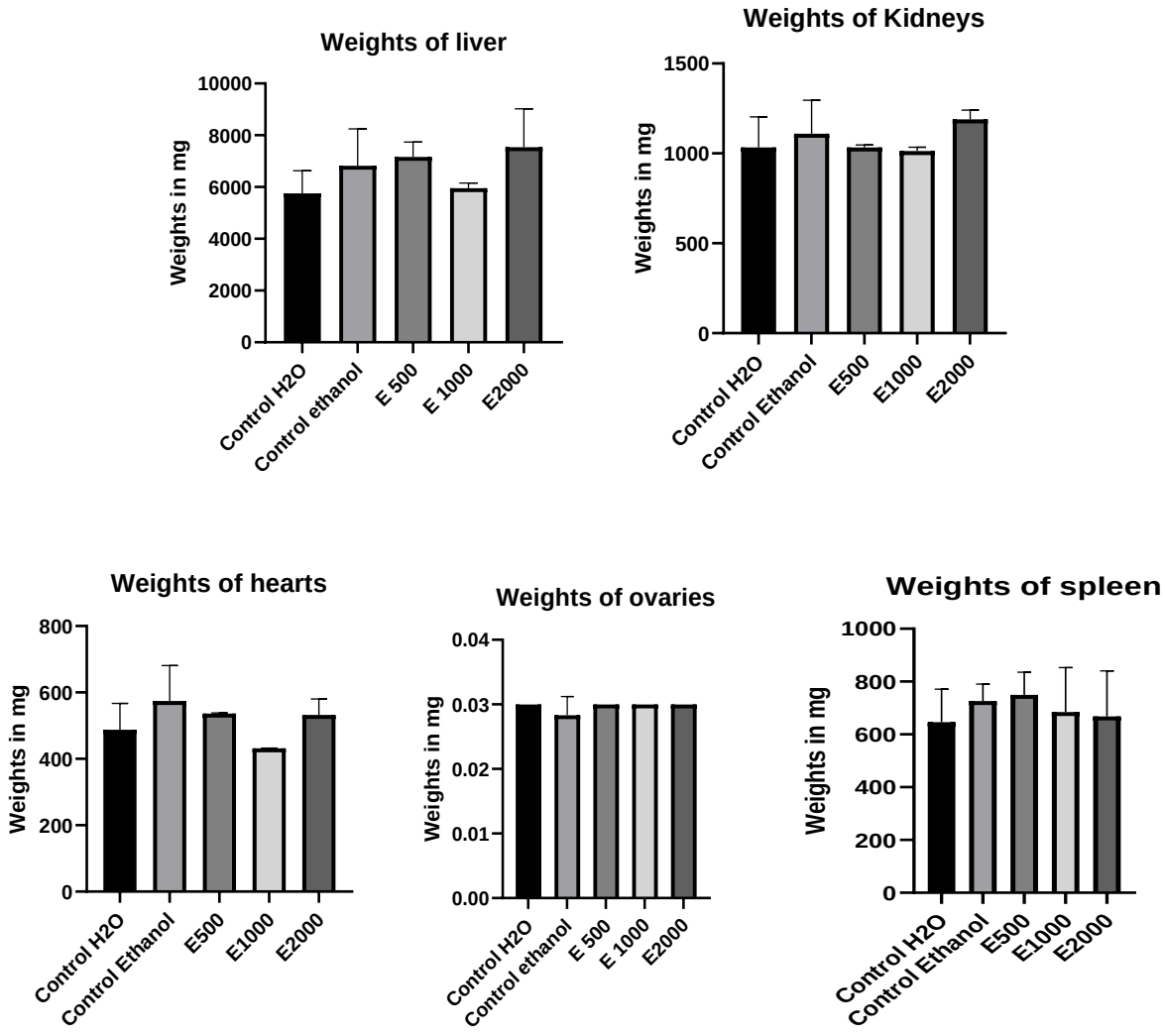

Figure 3: Visceral organ weights. The data represents the Mean ± SD for each group of rats, n = 3 (number of animals per group) at $p<0.05$

Monitoring the estrous cycle

The various stages of the estrous cycle (Proestrous, estrous, metestrus and diestrus) were monitored during the study period. The number of 5-day cycle ranged from 4-5 in the control groups while those in the test groups ranged from 3-4 cycles as shown in Figure 4.

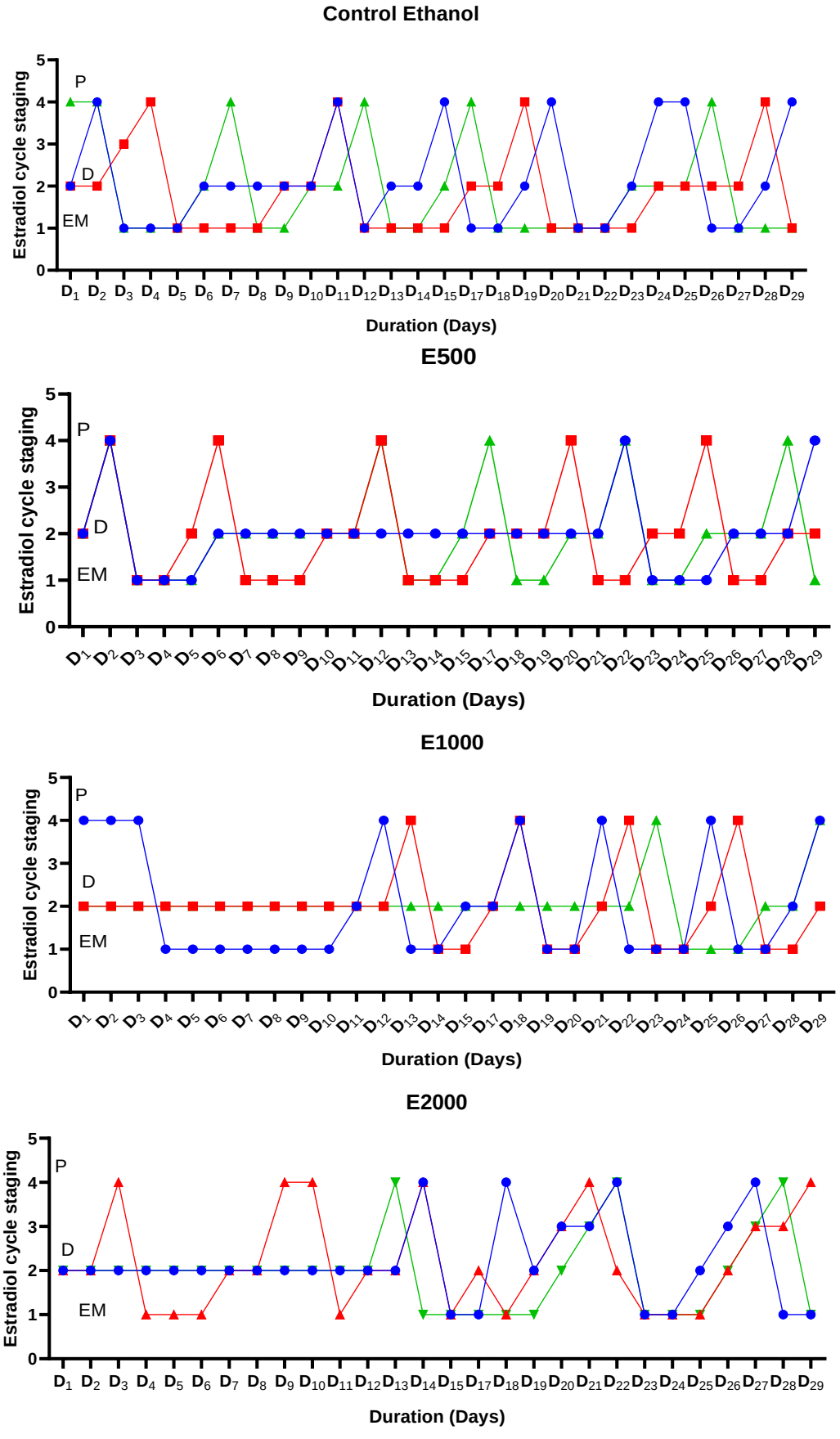

Figure 4 a, b,c,d: Number of estrous cycles in the various groups (P=proestrous, D=diestrous, EM=estrous/metestrous). The red, blue and green lines represent the three animals per group.

Histopathological findings

Histopathological examination of the liver, spleen, heart, ovaries and kidney revealed normal morphological structures in control groups and treated groups without signs of vascular or inflammatory changes (Figure 5). Follicles can be classified as either primodial, primary, secondary, or Graafian according to the presence and size of an antrum. We observed a well visible nucleus, an organized structure of granulosa cells and follicular antrum in the test and control groups. Also, a well deliminated uterine lining was observed surrounded by a myometrium and endometrium (Figure 6)

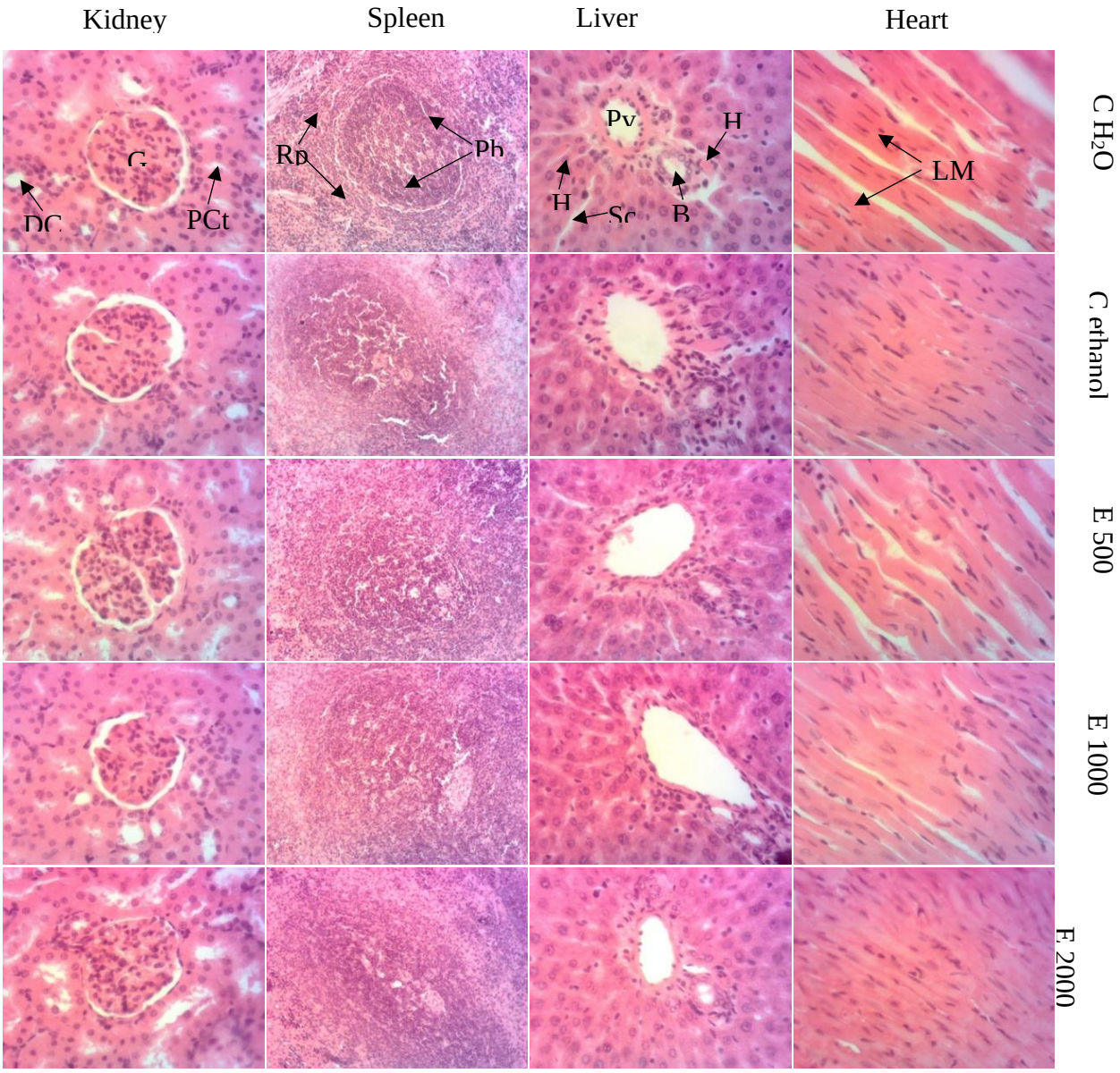

Figure 5: Architecture of kidney, liver, heart (200X, Hematoxylin-eosin) and spleen (100X, Hematoxylin-eosin). Pv = : portal vein; Bc = : biliary canaliculus; H = : hepatocyte; Sc = : sinusoidal capillary; Ha = : hepatic artery; Gl = : glomerulus; DCt = : distal convoluted tubule; PCt = proximal convoluted tubule; Wp = white pulp; Rp = red pulp; LMF = longitudinal muscle fibers.

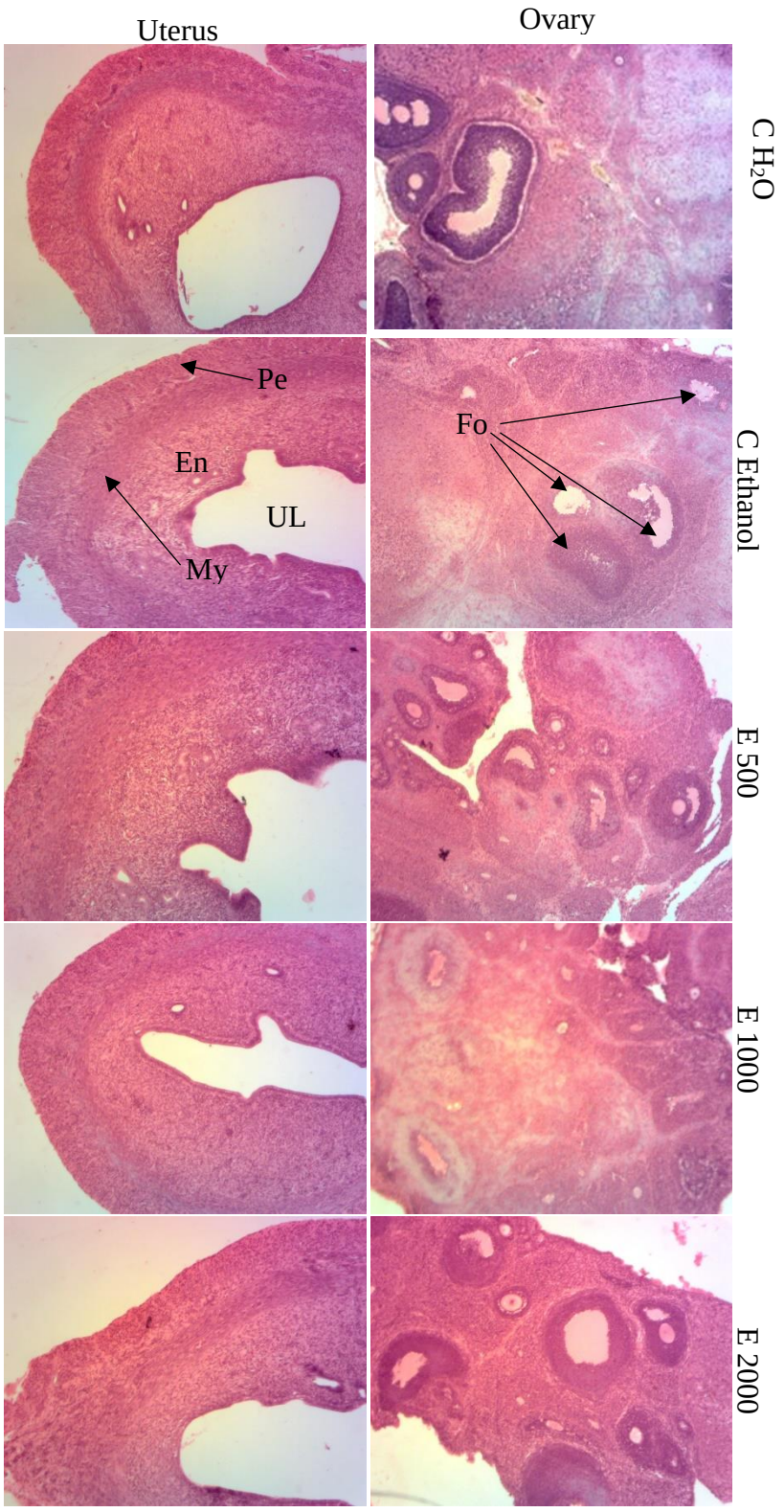

Figure 6: Architecture of uterus and ovary (40X, Hematoxylin-Eosin). UL = Uterine lumen; En = Endometrium; My = Myometrium; Pe = Perimeter; Fo = follicles at different stages of development

Effect of aqueous extract of *T. grandis* leaves on biochemical parameters

Following administration of the extract at varying doses for the 28 day study period, no significant changes were observed in the biochemical parameters of the test groups to the control groups. However, we observed a markedly elevated non-significant level of liver ALT at the dose of 2000 mg/kg body weight. Also, we observed a dose dependent increase in cholesterol levels in the treated groups when compared to the control (Table 1)

Table 1: Effect of aqueous extract of *T. grandis* leaves on liver, Kidney function markers and lipid profiles of animals (mean ± SEM)

	Liver			Serum				
Groups	**ALT (IU/dl)**	**AST (IU/dl)**	**ALP (IU/dl)**	**Total Protein (g/dl)**	**Glucose (mg/dl)**	**Creatinine (mg/dl)**	**Cholesterol (mg/dl)**	**Uric Acid (µmol/L)**
Control H_2O	93.7±44.8	58.2±14.6	374±107	6.05±0.164	103±9.94	3.44±0.29	117±9.67	107±11.6
Control EtOH	59.9±15.2	34.3±17.1	318±33.6	6.23±0.355	90.1±4.48	3.56±0.11	119±2.75	75±37.4
E500	187±41.1	43.4±17.7	387±3.15	6.11±0.06	95.3±8.94	3.0±0.39	136±5.30	107±23.3
E1000	57.6±16	54.1±13.6	384±26.7	6.01±0.022	81.1±7.22	2.67±0.19	134±5.02	94.6±8.57
E2000	1311±68	100±6.55	439±11	5.92±0.09	73.3±13.3	2.0±0.77	127±0.697	117±31.6
Range	**13-56**	**34-109**	**95-611**	**6.3-7.3**	**44-208**	**0.25-3.09**	**41-126**	**75-160**

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP) (Reference for serum chemistry ranges for rat: Chen et al, 2006; Loeb and Quimby et al, 1999)

Effect of aqueous extract of *T. grandis* leaves on the haematological parameters

There were no statistical significant differences observed in the blood cells in the test and control groups. However, there was a decrease in the white blood cells, haematocrit and haemoglobin concentration at the dose of 2000 mg/kg BW (Table 2).

Table 2: Haematological parameters in the experimental animals after 28 days of extract administration

Groups/ Blood cells	Control EtOH	Control H_2O	E500	E1000	E2000	Range
White blood cells	3.98±1.98	6.85 ± 2.2	4.48 ± 0.2	8.65 ± 3.7	2.17 ± 0.11	4-120*10^9/L
Lymphocytes	2.7±1.27	4.57±1.89	3.25 ± 0.12	7.47 ± 3.4	1.44 ± 0.03	0,8-7*10^9/L
%Lymphoctes	69.4±2.56	62.9±5.71	72.6 ± 0.433	79.5 ± 5.34	56.9 ± 10.7	19.1-60%
Monocytes	0.45±0.14	1.08±0.3	0.64 ± 0.01	0.39 ± 0.01	0.43 ± 0.01	0.1-1.5*10^9/L
% Monocytes	13.4±2.37	16.3±1.78	14.3 ± 0.41	9.95 ± 4.24	17.5 ± 3.37	4.5-15%
Granulocytes	0.83±0.59	1.19±0.01	0.59 ± 0.06	0.795 ± 0.3	0.25 ± 0.09	2-8*10^9/L
%Granulocytes	17.2 ± 4.9	20.8±5.12	13.2 ± 0.84	10.6 ± 1.1	9.1 ± 5.2	50-70%
Red blood cells	6.4 ± 0.6	7.21±2.25	21.9 ± 8.7	7.3 ± 0.18	5.2 ± 1.3	3.5-5.8*10^{12}/L
Heamoglobin	12.1 ± 1.24	11.4±2.48	11.9 ± 0.38	13.6 ± 0.12	9.35 ± 2.17	11-17 g/L
Hematocrit	34.7 ± 3.86	40.4 ± 13.1	28 ± 6.87	42.8 ± 0.94	28.7 ± 7.25	34-53%
Mean corpuscular volume (MCV)	54.5 ± 0.84	55.4 ± 1.45	50.2 ± 4.5	58.6 ± 0.12	55.3 ± 0.58	80-100 fL
Mean corpuscular hemoglobin (MCH)	19 ± 0.31	16.9 ± 1.92	23.9 ± 3.29	18.7 ± 0.29	18.5 ± 0.32	27-34g/L
Mean corpuscular hemoglobin conc (MCHC)	34.8 ± 0.4	30.7 ± 3.8	50.6 ± 11.1	31.9 ± 0.43	33.3 ± 0.92	32-36g/dL
RDW-SD	26.5 ± 1.2	29.5 ± 1.43	24.1 ± 5.43	30.9 ± 1.29	29.1 ± 1.99	35-56 fL
RDW-CV	11.9 ± 5.9	13 ± 6.44	19.2 ± 0.43	18.7 ± 0.09	10.1 ± 5.73	11-16%
Platelets	569 ± 13.9	398 ± 13.0	573 ± 21.5	550 ± 47.3	512 ± 83	156-342*10^9
Thrombocytes %	1.21 ± 1.04	0.5 ± 0.14	0.35 ± 0.13	0.33 ± 0.03	0.44 ± 0.01	0.16-0.36%
Mean platelet volume	5.5 ± 0.36	6.5 ± 0.4	5.95 ± 0.03	5.9 ± 0.01	5.9 ± 0.12	6.5-12fL

Estrogenic activity of *T. grandis* in serum and ovaries

T. grandis demonstrated a dose dependent increase in Rat 17-β-estradiol (E2) in the serum and ovaries of the test groups when compared to their concurrent controls using a pre-coated competitive- ELISA detection Kit. However, the levels of expression of 17-β-estradiol (E2) in the test groups' serum was less than that in the ovary when compared to the control groups (Figure 6a and b). Using Spearman's correlation we obtained a significant ($P < 0.01$) negative perfect correlation ($r = -1.0$) between the total cholesterol levels and ovarian estradiol. Meanwhile a non-significant negative Spearman's correlation ($r = -0.3$) was observed between total cholesterol and serum estradiol.

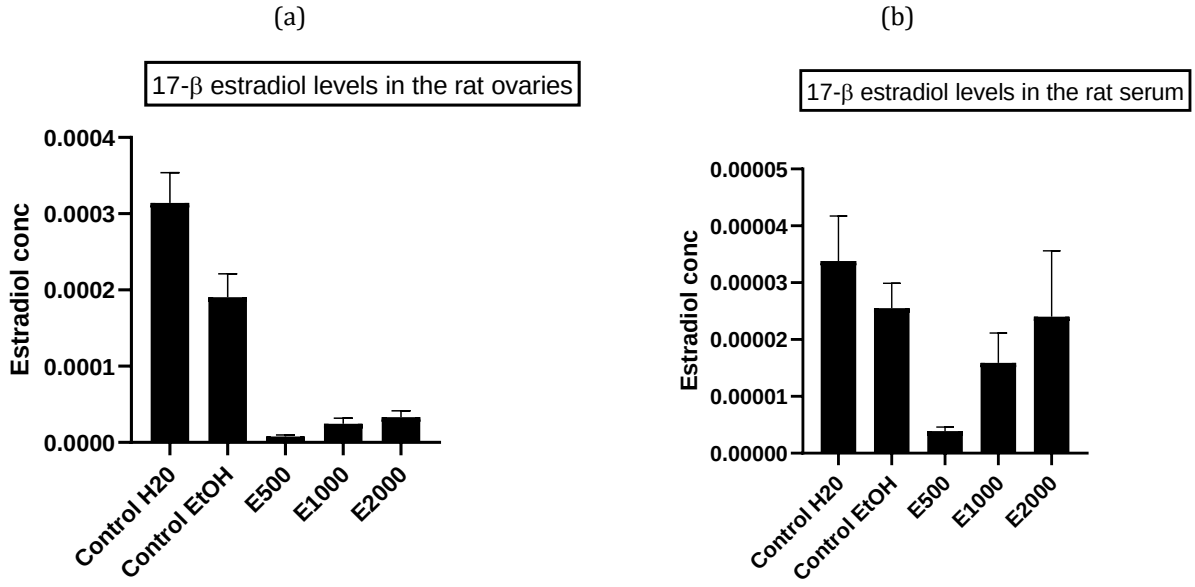

Figure 7 a & b: Effect of *Tectona grandis* on 17-β-estradiol (E2) levels in the test versus control groups

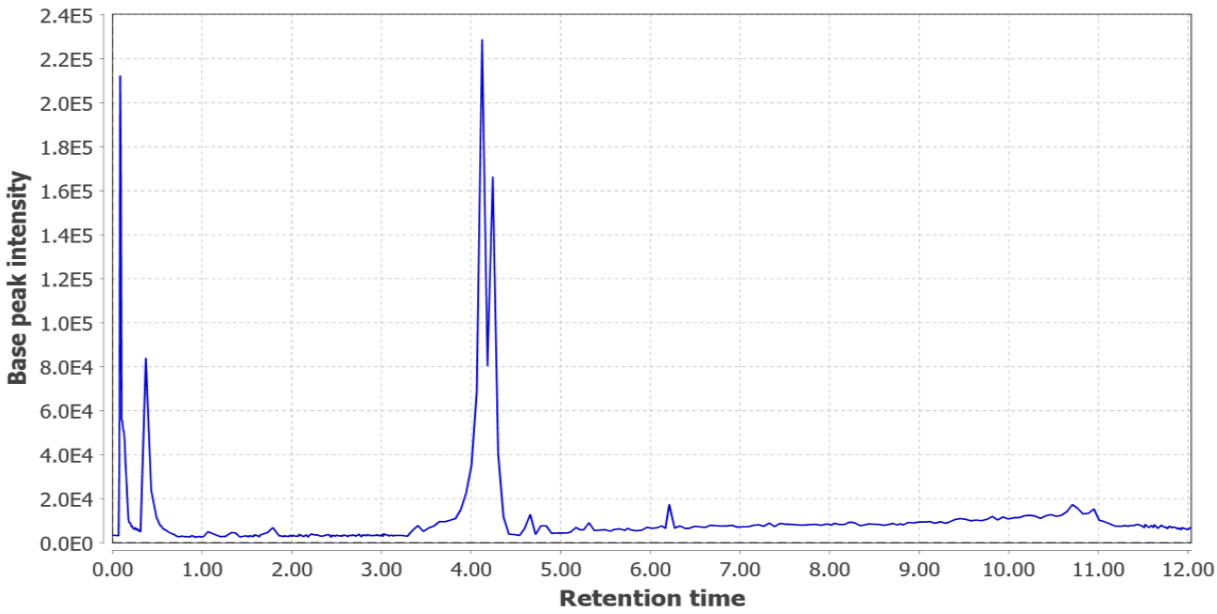

Figure 8: Total ion chromatograms of *T. grandis* leaves extract

Table 3: Annotated compounds from the total ion chromatograms of *T. grandis*

Rt	Acquired m/z	Adduct	Exact mass	Formula	Annotated Compounds
0.09	379.1329	$[M-H]^-$	379.1909	$C_{24}H_{28}O_4$	Diethylstibesterol diproprionate (**1**)
4.12	623.1748	$[M-H]^-$	624.1878	$C_{50}H_{24}$	Nd
4.24	621.1652	$[M-H]^-$	622.1722	$C_{50}H_{22}$	Nd
4.86	285.0161	$[M - H]^-$	285.1126	$C_{17}H_{18}O_4$	Yateresinol (**2**)
5.34	327.1928	$[M - H]^-$	327.0868	$C_{18}H_{16}O_6$	Tectonoelin A (**3**)
5.35	395.1833	$[M - Cl]^-$	395.1261	$C_{20}H_{24}O_6$	Lariciresinol (**4**)
5.78	315.0365	$[M - Cl]^-$	315.0635	$C_{14}H_{16}O_6$	Zhepeiresinol (**5**)
5.92	193.0613	$[M - Cl]^-$	193.0056	$C_{10}H_6O_{12}$	1,4-naphtoquinone (**6**)
10.31	409.2884	$[M - Cl]^-$	409.1058	$C_{20}H_{22}O_7$	8-hydroxypinoresinol (**7**)

T. grandis contains diverse metabolites including quinones, terpenes, flavonoids and lignans. Compounds **3**, **4** and **5** were previously reported from *T. grandis* leaves [11]. Yateresinol **(2)** was reported in *Libocedrus Yateensis* [21]. 8-hydroxypinoresinol **(7)** isomer, 1-hydroxypinoresinol was previously reported from *T. grandis* leaves [11]. 1,4-naphtoquinone **(6)** was isolated from *Juglans* species [22]. Diethylstibesterol diproprionate (**1**) is the dipropionate ester of a synthetic non-steroidal form of oestrogen [23].

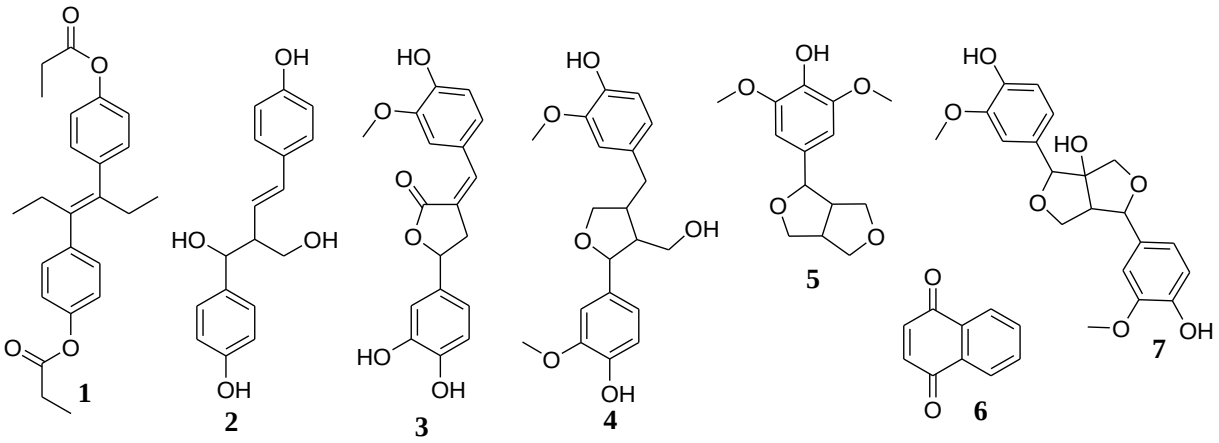

Figure 9: Structure of annotated compounds **1**-**7** from *T. grandis* total ion chromatograms.

DISCUSSION

The reproductive period and estrous cycle of rats begin about the 26th day after birth with the opening of the vagina, which is about 10 days before vaginal cornification [19]. An essential secondary characteristic and a predictor of puberty in rats is the apoptosis mediated vaginal opening (occurs during first ovulation) [18, 24] which is associated with an increase in estradiol concentration. The aim of our study was to investigate estrogenic effects of *T. grandis* on the female reproduction, while assessing their reproductive toxicity for the development of new hormonal therapies. Our results show a dose dependent non-endogenous effect on estradiol concentration in the ovaries and serum in the test groups when compared to controls. Interestingly, the levels of this hormone was lower in the serum compared to ovary meaning the phytoestrogens in the plant could induce their effects on the ovarian estrogen receptors competing for the same binding site like the endogenous estrogen. In our study, we also observed a diminution of one cycle and prolonged diestrus states in some of the test animals compared to the control groups. Blood samples and ovary sections were obtained from the animals at their proestrus state where estradiol is secreted maximally. The negative correlation between cholesterol and estradiol was expected given that cholesterol is the precursor of estradiol production.

Assessment of the estrous cycle in experimental animals is a useful measure of the integrity of the hypothalamic-pituitary-ovarian axis and the functioning reproductive status of the female reproductive system [18]. The effects of drugs and chemicals on reproductive function [25] can also be assessed on the disruption of the intact morphology, cytology and histology of reproductive organs and alteration in the duration of the estrous cycle phases. Previous phytochemical studies on *T. grandis* have led to the isolation of several lignans [11, 26, 27]. Lignans including 8-hydroxypinoresinol, lariciresinol, tectonoelin A, yateresinol and zhepeiresinol have been detected in the studied extract by LC-MS analysis and the repeated dosing regimen of the extract for 28 days was evident with the dose dependent increase in levels of estradiol. Lignans are phytoestrogens, estrogen-like compounds derived from plants, with structures similar to that of the

endogenous 17β-estradiol. Phytoestrogens are able to bind alpha and beta receptors of estrogen affecting the expression of certain genes including the induction of sex hormones that bind to globulin and inhibit aromatase [28]. Alpha estrogen receptors act in cell proliferation, whereas beta receptors are responsible for cell apoptosis [29]. Endogenous estrogen levels also affect the activity of phytoestrogens. For example, lignans compete with endogenous estrogen to bind to estrogen receptors so that they can inhibit estrogen activity. At menopause, endogenous estrogen levels production by the ovaries is low and, in this condition, the lignans also tend to compete better with the endogenous estrogens [30]. Lignans exist in the plant as glycosides stored in the vacuole, and are converted to active phytoestrogens by microflora in the proximal colon [31]. Thus, the activity of intestinal microflora is critical, in the metabolism of plant extracts containing lignans. The hormone-dependent diseases such as cancers, atherosclerosis, and coronary heart disease are all associated in one way or the other with sex hormones and their metabolism, and intake of phytoestrogens does have some physiological effects in humans related to hormone regulation, but like hormones, the benefits depend on the stage of life [32].

While assessing the estrogenic activity of the plant, we did not observe a significant difference in the body weights of the experimental and control animals. This also corroborated with organ weights where no significant difference was observed in the test and control groups thus indicating that the plant is safe to be administered for 28 days study period. This was further confirmed by the histopathological findings which showed no abnormalities in the test and control groups of the visceral organs. The marked increase in liver function enzyme (ALT) levels at the dose of 2000 mg/kg body weight could be due to elevated doses of the extract (medicinal product) and not liver damage given that this increase was not significant and histopathological findings of the liver did not indicate any abnormalities. For the hematological analysis, we observed non-significant decrease in the white blood cells, haematocrit and haemoglobin concentration at the dose of 2000 mg/kg BW which indicates the hematopoietic effect of this plant at higher doses. The function of the white blood cells is to protect the body from infection by foreign organisms, while the red blood cells boosts the immune system by providing nourishment and oxygen and the platelets protect blood vessels from damage as well as initiate repair of these vessels during trauma. The MCV and MCH give the volume and weight of the hemoglobin in each red blood cell while the MCHC gives a valuable indicator of hemoglobin deficiency [33]. The decrease in MCV and MCH values in all the groups indicated low weight of the hemoglobin. However, the MCHC values were all normal and conclusive of no hemoglobin deficiency.

CONCLUSION

T. grandis ethanol leave extract is safe at the doses tested and contains phytoestrogens which can be exploited as estrogenic alternatives to normal endogenous estrogens for the treatment of menopause and infertility. Because of their structural similarity to estrogen, phytoestrogens can bind to estrogen receptors and exert their effects depending on the concentration and tissue considered. As such, phytoestrogens can alter endogenous hormonal activity, ovarian hormone profiles, and fecundity.

Author Contributions

Adela Ngwewondo: Conceptualization, Data Curation, Investigation, Methodology, Resources, Visualization, Writing of original draft **Ferdinand Lanvin Edoun Ebouel :** Data curation, Investigation, Methodology, Resources, editing **Théodora Kopa Kowa:** Resources, Conceptualization, Investigation**, Lauve Rachel Tchokouaha Yamthe:** Investigation, methodology**,** review and editing, **Stephanie Guetchueng Tamdem:** Investigation, editing **Armelle Tchamgoue Deutou:** Methodology, editing**, Protus Arrey Tarkang:** Project administration, validation**,** review and editing, **Lenta Bruno**: Investigation, validation **Gabriel Agbor Agbor:** Conceptualization, Project administration, validation, review and editing

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List of Abbreviations:

JIRB: Joint Institutional Review Board for Animal and Human Bioethics
LD50: Lethal dose
BW: Body weight
HE: hematoxylin-eosin
ALP: alkaline phosphatase
AST: Aspartate aminotransferase,
ALT: Alanine aminotransferase
E2: 17-β-estradiol
MHT: Menopausal hormone therapy

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